THE VASODILATOR ACTION OF HISTAMINE AND OF SOME OTHER SUBSTANCES.

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CONTENTS.

THE immediate object of the experiments with which this paper deals was a more complete analysis of the vasodilator effect produced, in some species of animals, by small intravenous doses of the substance which we have here called histamine. In earlier papers P. P. Laidlaw and one of us(1) referred to it by its chemical name, β-Iminazolylethylamine (abbreviated for convenience to β-I.), being unwilling to coin a new name for a substance long known, though its great physiological activity and occurrence in nature were at that time newly discovered. Several later investigators of its action, however, have used for it the name "histamine," which is so obviously appropriate for this amine derived from histidine, that we have adopted it here.

The most puzzling feature of the action of histamine, as described by Dale and Laidlaw, was the fact that this intense stimulant of plain muscle caused in the carnivora, and also in the monkey and the fowl, a

profound fall of the arterial blood-pressure in the major circulation, due apparently to pure vasodilatation. Plain muscle from any organ of the body of various species was found to be stimulated to intense tonus. A solitary and curious exception has since been found by Guggenheim(2) in the plain muscle of the rat's uterus. In some species, such as the rabbit, histamine caused a vasoconstrictor rise of arterial pressure, such as its property of stimulating plain muscle would lead one to expect. Even in the cat a vasoconstrictor action could be detected in the pulmonary circulation, and in the kidney; but the general effect in this species was vasodilatation, so long as the injection was made into the blood stream of the living, anæsthetised animal. In surviving organs of the cat, on the other hand, perfused with defibrinated blood, histamine was found always to cause simple vasoconstriction, as demonstrated by the record of venous outflow and by the plethysmograph. Later observers have shown that isolated strips of artery, from various organs, are always stimulated to increased tonus by application of histamine.

Dale and Laidlaw considered and tested the more obvious possibilities of explaining this vasodilator action, paradoxically produced by a general stimulant of plain muscle, but left the problem unsolved. The numerous papers dealing with the action of histamine which have since appeared have not materially affected this position, being concerned, for the most part, with detailed investigation on particular organs of an action in conformity with the general type indicated. An attempt has been made, however, by Mautner and Pick(3) to account for the discrepancy in action with which we are concerned, and we shall deal with this later.

The immediate impulse to a renewed investigation of this anomalous vasodilator action of histamine was furnished by the observation that this substance in larger doses produces, in the cat or dog, a condition of profound shock, having many of the features which recent work has associated with shock of traumatic origin. This shock-like effect of large doses of histamine has been described by E. Mellanby(4), who investigated especially the conditions determining its production by absorption of histamine from the bowel. A preliminary account of a further analysis of the condition by Dale and Laidlaw(5) has already been published, and fuller details will be given in a forthcoming paper. The shock-like condition caused by large doses seemed to be definitely associated with the vasodilatation which small doses produce in an evanescent form; the one seemed to result from a prolongation and intensification of the other, and both types of action, if indeed they were separate, were observed

only in certain and the same species. It seemed to us, therefore, that a further attempt to analyse and explain the vasodilator action of small doses was a necessary part of the investigation of the shock-like conditions produced by larger doses, which in turn might be expected to throw light, if only by inference, on the nature of the circulatory defect characteristic of traumatic shock and allied conditions, at present of such pressing interest and importance.

Incidentally the opportunity has occurred of examining, in comparison with that of histamine, the vasodilator effects of certain other substances, in particular those of minute doses of adrenaline and of acetyl-choline. Our experiments lead us to reject the conception recently put forward by Hartmann and Fraser (6), according to which the vasodilator effect produced by very small doses of adrenaline in the cat and dog is an indirect effect, due to action on the nervous centres. In the case of acetyl-choline we give additional evidence concerning the vasodilator action described by one of us (7) (H. H. D.), and recently confirmed by Reid Hunt (8), who first described the intense depressor action of this substance. We find that this action can be demonstrated in conditions under which the vasodilator effect of histamine no longer appears, and the difference furnishes an important link in our evidence concerning the mode of action of the latter substance.

I. Methods.

All our experiments have been made upon cats, except where otherwise stated. Ether has been the anæsthetic regularly used, chloroform being sometimes used for the preliminary induction. In the experiments in which observations have been made on denervated organs the nerve sections have been carried out under ether, with full precautions against sepsis. After the requisite period for degeneration the main observation has been made under ether in the usual manner. Occasionally the progress of sensitiveness to histamine has been tested, during the degeneration of nerves to a limb, by injecting a small dose of histamine into the ear-vein and directly observing the vasomotor changes in the unpigmented pads of the feet; or, again, such an interim test has been made with the aid of plethysmographs on the legs, the animal being then immobilised by full ether anæsthesia and injections made into the ear-vein or into the jugular vein exposed aseptically by a very small skin-incision. After such an interim experiment, the animal has been allowed to recover from the anæsthetic, but after the main experiment, involving

dissection for the record of blood-pressure, etc., the animal was always killed while still anæsthetised.

Our experiments fall into two groups: those made on the animal with natural circulation, and those made on surviving organs perfused artificially. In experiments of both kinds the plethysmographic method was frequently employed. For the viscera we used air-oncometers of the usual type, shaped out of gutta-percha, and closed by glass lids made tight with vaseline. For the limbs we used cylindrical glass plethysmographs filled with warm water (or with saline in experiments on exposed muscle). The method of making a water-tight junction between the skin of the limb and the end of the plethysmograph deserves mention, as we have found it unusually convenient and trustworthy.

The glass cylinder is made considerably wider than the thickest part of the limb to be enclosed, and the water-tight junction is, as usual, effected by use of a suitable cuff of thin rubber. A section of inner tube

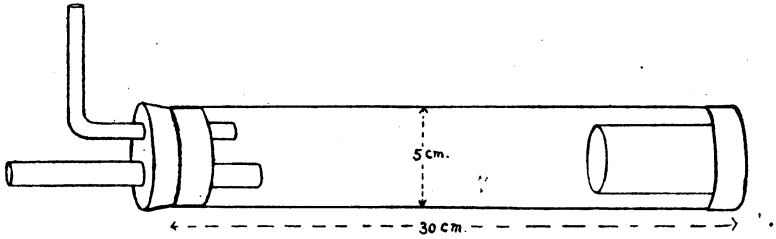

Fig. 1.

from a bicycle tyre serves well for the legs of most cats. The only point of novelty in our use of the cuff is that we invaginate its free portion into the open end of the glass cylinder, within which it forms a secondary, short elastic tube, separated from the glass by an annular space, a centimetre or so in breadth (Fig. 1). The rubber is well softened with vaseline, and the limb, at the level at which the connexion is to be made, is well anointed with the same substance, which is thoroughly incorporated with the fur. The plethysmograph, with its invaginated cuff, is simply pushed onto the limb, and an air-tight fit is usually secured immediately. If the limb is very thin it can be built up to the requisite thickness with vaseline-soaked cotton-wool, but this is seldom needed. When the junction has been tested the cylinder is filled, except for a small air-space at its distal end, with warm water. The water fills the annular space between glass cylinder and rubber cuff, the warm water softens the vaseline, and the small hydrostatic pressure, produced by giving the

apparatus a slight upward inclination, keeps the rubber gently pressed against the vaselined fur. The plethysmograph is then fixed in position by a condenser-clamp and the temperature of the water in it kept constant by means of a carbon filament glow-lamp placed near to it. When once this form of plethysmograph has been satisfactorily applied,—a procedure which seldom occupies more than a few minutes—it gives a very satisfactory safety from leakage without any undue pressure on the limb by the cuff. The leg can be raised with the plethysmograph to any required angle, even to the vertical, and the cylinder of the plethysmograph can be displaced along the length of the leg through several centimetres, without a leak occurring.

As volume-recorders we have throughout used small Brodie's bellows, covered with the prepared peritoneal membrane sold for surgical use as "Cargile membrane." Recorders made with this membrane, when kept supple by frequent application of dilute glycerine, have remained in almost daily use for months without leaking or giving trouble. For the perfusion of surviving organs we should have preferred to use an adjustable pump, such as that recently described by one of us (A. N. R.) with C. K. Drinker(9). Recent conditions making the construction of this or any suitable form of pump for us impossible, we have used a relatively simple system of perfusion under hydrostatic pressure, which has sufficed for most of our purposes. The adjustable pressure was obtained by slinging a Marriotte-bottle from the ceiling by a cord passing over a pulley. The outflow tube was connected by a long rubber tube with one end of a glass worm immersed in a water-bath. The temperature of the water was kept approximately constant by a long cylindrical glow-lamp with carbon filament, which could be inserted to any required depth into a metal tube, soldered into the side of the bath, and closed at its inner end. From the other end of the worm connection was made by rubber tubing to the arterial inflow-cannula, a glass T-piece being interposed for connection with a mercury manometer recording the perfusion pressure. The pressure was given a pulsatile character by the rhythmical compression of the rubber tube between the worm and the cannula by a small hammer driven by an electro-magnet. The electro-magnet was actuated by the house-current with a lamp resistance, the circuit being momentarily made with the desired rhythm by closure of a brass contact key by a rotating vulcanite cam.

As perfusion fluids we used chiefly hirudinised cats' blood, and oxygenated Locke-Ringer's fluid to which the requisite viscosity was imparted by adding 6 p.c. of gum arabic freed from calcium, according

to the recent indications of Bayliss(10), who showed that such an addition also supplied the required amount of osmotically active colloid. In two experiments we used a lower proportion of gum in the Ringer solution and suspended washed corpuscles from cats' blood in the mixture. In one case we obtained a result before agglutination of the corpuscles interfered with the perfusion; in the other case the presence of the gum caused such rapid agglutination of the corpuscles that the experiment had to be abandoned. Bayliss has observed that the red corpuscles of the cat, unlike those of man, are agglutinated by gum-arabic.

The rate of outflow from the cannula in the vein leading from the perfused organ was measured in all experiments. In earlier experiments we used an electrically recording tilter for automatic registration of the rate, but more recently we allowed the outflowing blood or fluid to run into a measuring burette and recorded the completion of each c.c. by an electric signal. From time to time the fluid collected from the vein was returned to the Marriotte-bottle. The lack of apparatus to effect this return automatically necessitated the use of rather large volumes of fluid, to prevent too frequent interruptions of the record; so that when blood was used we generally employed the whole blood of two cats for an experiment.

II. Mautner and Pick's Theory.

The theory of Mautner and Pick, already mentioned above, is stated in what appears to be a preliminary communication, since no quantitative details are given. Their experiments were carried out on artificially perfused livers and lungs of various species. They found that histamine and other substances belonging to the group which they denominate "shock-poisons" produced obstruction to the perfusion through the portal circulation of the cat and dog. In these species, therefore, they regard the fall of arterial pressure as due to the obstruction of blood-flow through the liver. Since the liver expands as the outflow through the hepatic veins diminishes, they locate the obstruction in the capillaries opening into the intralobular veins. Such part of the depressor action as is not explained by this obstruction in the liver—presumably the whole of the depressor effect in the monkey, in which they detected no impediment to the liver-circulation—they attribute to constriction of the pulmonary vessels. The possibility of the fall of systemic arterial pressure in the cat being a result of vasoconstrictor action on the pulmonary vessels was considered by Dale and Laidlaw,

who showed that such an explanation was inconsistent with the facts. Among other points of evidence against it they mentioned the fact that the fall of arterial pressure has a shorter latency when histamine is injected into the arterial blood-stream than when it is injected into a vein.

In attributing such general significance to the obstruction in the liver of the cat and dog, as an explanation of the depressor action in these species, it seems to us that Mautner and Pick have too readily assumed that all the effects of all doses of the "shock-poisons" can be explained by one feature of their action in massive doses. Nobody who has watched the exposed abdominal viscera of a dog after a sufficient dose of peptone has been injected to produce "peptone-shock," or during the closely similar "anaphylactic-shock," can be in doubt as to the large part played in the effect by obstruction to the circulation through the liver.

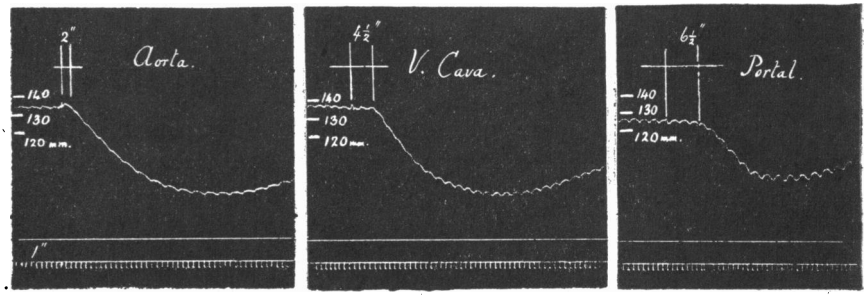

Fig. 2. Effects of intra-aortic, intracaval and intraportal injections of 0·01 mgm. histamine.

The appearance of the enormously swollen liver, tense and dark with blood, of the turgid mesenteric veins and injected coils of intestine, shows clearly that an important part of the blood of the whole body has been temporarily arrested in the splanchnic area. How far such an effect enters into the production of "histamine-shock" we shall consider in a later paper. For the present we are concerned with the rapidly evanescent depressor effect of small doses, which cannot be explained on these lines. The plethysmographic records, with which we deal further in a later section, furnish clear evidence against Mautner and Pick's view; but we may first note two other directions in which decisive evidence against it may be found.

Time relations of the depressor action on injection into different parts of the circulation. If Mautner and Pick's assumption were correct the latency of the depressor action in the cat and dog should be shortest

when the injection is made into the portal vein. We compared the latencies of the action of small doses of histamine with injections into the lumen of the portal vein by a needle inserted through the splenic vein, into the aortic arch by a needle inserted through the central stump of the tied carotid artery, and into the inferior vena cava by a needle inserted through a renal vein. The injection was signalled by a sharp compression of the thick-walled rubber tube connecting the arterial cannula with the manometer, the necessity of accurate alignment of a separate signal-record being thus obviated. Fig. 2 shows a record from such an experiment. It is obvious, even without measurement, that the latency is least with arterial, intermediate with caval and longest with portal injection. It will also be obvious that, though the same dose is given in each case, the fall of pressure produced by intracaval injection is conspicuously deeper than that following intraportal injection, presumably because part of the histamine given by the latter route is destroyed in the liver before reaching the general circulation. The actual duration of the latency of the effect of an injection will necessarily vary with the rate of the blood-flow, so that the effect of an intra-arterial injection given when the arterial pressure is high will appear more promptly than that of an injection given when the pressure is lower; but even when conditions of flow are practically constant, the effect is consistently later in appearance with intraportal than with intracaval injection. Histamine, therefore, produces its depressor action at some point reached earliest by blood in the arteries, later by blood in the systemic veins, and later still by blood in the portal vein. The action cannot, therefore, be located in the liver or the lungs.

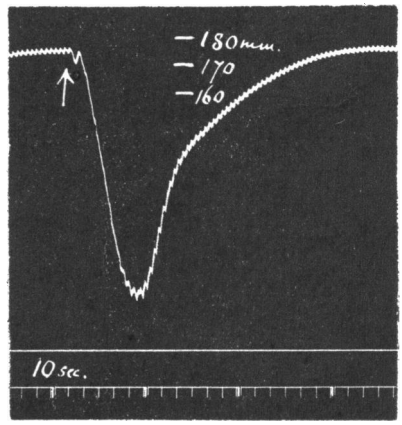

Fig. 3. Effect of 0·025 mgm. histamine on blood-pressure of eviscerated cat.

Action on the eviscerated animal. Further evidence against the view that events in the liver play an important part in the depressor action may be found in the fact that it is produced, without noteworthy impairment, after the vessels to the stomach, intestine and liver have all been ligatured and the whole of the abdominal portion of the alimentary canal has been removed. Fig. 3 shows the fall of blood-pressure produced

by 0·025 mgm. of histamine in an animal thus eviscerated, and with the liver therefore excluded from the circulation.

III. Plethysmographic records from the animal with normal circulation.

Since Mautner and Pick's suggestion failed to afford an explanation of the anomalous vasodilator action, we returned to a more detailed investigation of the effect as demonstrated by plethysmographic records, with special reference to hitherto unexplored possibilities of its dependence on the integrity of the nervous connexions of the blood vessels. Such possibilities, as will be seen, were soon excluded, but in the process of their consideration we had taken the opportunity of examining the nature of the vasodilator action of other substances. We ultimately selected two for special study—adrenaline, because in the failure to demonstrate its vasodilator action by perfusion and in the limitation of this action to certain species (it has only been observed hitherto in the carnivora) it presents some points of resemblance to histamine; and acetyl-choline, because it has a more typical vasodilator action, demonstrable even by perfusion of an organ with cold Ringer's solution (Dale(7), Hunt(8)), occurring in all species on which the observation has been made, and so rapidly evanescent that small doses can be given repeatedly without permanently affecting the condition and responsiveness of the animal. The comparison of the effects of these substances with those of histamine under varying conditions yielded some highly suggestive results, the significance of which could be further tested by experiments on artificially perfused organs.

Many of the experiments now to be described deal with a comparison of effects in the two hind limbs. Care was taken in such cases to insert equal parts of the two limbs into the plethysmographs. The two recording bellows were of identical dimensions, but it unfortunately happened that one was covered with a slightly thicker membrane, so that its movement was rather less prompt and delicate than that of the other. In comparative records the more sensitive of the two bellows always writes the upper of the two records, except when the contrary is stated, and small differences in the direction of greater rapidity and extent of movement recorded in the upper line must not be regarded as significant. It is only to major and unmistakeable differences in this direction that we attribute importance in our argument.

(i) *Direct nature of the vasodilator effects.* We must first exclude the possibility that the action of histamine, and of the other vasodilator

substances with which we deal, may be due to an action on the central nervous system or on some more peripherally placed nervous mechanism. The participation of the sympathetic nerve supply in the vasodilator action of histamine had already been excluded by Dale and Laidlaw, but they had not dealt with the possibility that the vasodilator effect might be due to antidromic impulses in sensory fibres, whether excited by stimulation of some part of the central nervous system, or by an axon reflex from sensory nerve-endings, of the type invoked by Bruce in explanation of the local vasodilatation produced by irritant substances. In the case of adrenaline, Hartmann and Fraser(6) as the result of experiments on the immediate effects of nerve-section and on the separate perfusion of an organ while adrenaline was injected into the general circulation, concluded that the vasodilator action was due to an action on the central nervous system. A study of their experiments suggested to us that their results were in part attributable to the use of too large doses, so that some of the dilator effects which they studied were depressor reflexes secondary to the primary pressor effects, and in part to a failure to appreciate fully the immediate effect of nerve section, in producing practically maximal vasodilatation in the affected limb for the time being. Since our own experiments had convinced us that these suspicions were correct, Gruber(11) has published experiments which satisfactorily demonstrate the peripheral nature of the vasodilator action of adrenaline on the vessels of skeletal muscles, by showing that it survives degeneration of the nerves to a leg, though it may be temporarily obscured by the immediate effect of their section. The fact that the dilator action of acetyl-choline can be demonstrated on an isolated organ perfused with a saline solution (Dale, Hunt) had established the fact that this substance produced its effect by peripheral action. There remained a possibility, though perhaps no great likelihood, that this action might be dependent on the integrity of peripheral endings of sensory nerves. Hunt apparently regards the effect of atropine, which abolishes the vasodilator action of acetyl-choline but leaves that of sensory nerve stimulation intact, as strong evidence against this suggestion.

All such possibilities are at once definitely set aside by the reaction to these substances of a limb completely denervated by degeneration of all its nerves. Fig. 4 shows the expansion of a cat's leg, 16 days after section of the sciatic and anterior crural nerves, in response to small intravenous doses of histamine, adrenaline and acetyl-choline, with the concomitant falls of arterial blood-pressure. It may be noted that, in

the case of adrenaline only, the expansion is followed by a distinct shrinkage of the limb. Such a result makes it certain that all three substances can produce vasodilatation by a direct action on the blood vessels, which is independent of the persistence of any nervous connexion; a further possibility of explaining the failure of histamine to dilate the vessels of the artificially perfused organ is thereby removed. An apparent elimination of any of these effects, as the immediate result of cutting the nerves to the organ under examination, as in the earlier experiments of

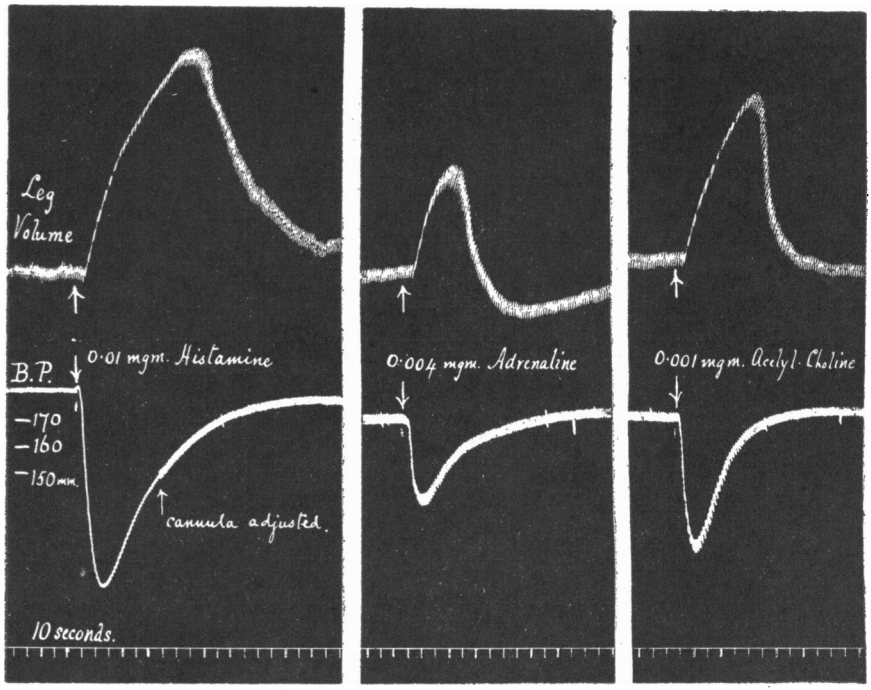

Fig. 4. Volume of leg completely denervated by degeneration; blood-pressure. Intravenous injections as indicated.

Gruber(12) and those of Hartmann and Fraser on adrenaline, must therefore find its explanation along other lines. Nevertheless a comparison of such immediate effects of section on the vasodilator responses to the three substances has yielded results which throw a good deal of light on our problem. We have also to consider whether the direct action, as shown by the denerved organ, accounts for the whole of the vasodilator action of these substances—whether the dilator effect is weakened, unaltered, or enhanced by degeneration of the nerves.

(ii) *Volume-changes in the normally innervated leg.* We must note, in the first place, that the hind leg, which is the organ on which nearly all our plethysmographic observations were made, shows remarkable irregularity in the changes of volume which accompany the general fall of blood-pressure produced by small doses of these substances, and in particular of histamine and adrenaline. Hunt describes the expansion of the hind limb with a small dose of acetyl-choline as likewise irregular, in comparison with that of the fore limb, but in our experience with very small doses of this substance (0·001 to 0·01 mgm.) the hind leg has very consistently shown a well-defined expansion, corresponding fairly closely with the fall of blood-pressure, the only complication being a slight preliminary shrinkage, which we regard as the passive effect of the dilatation starting earlier in organs nearer the heart. Our experiments on this point have all been made on the cat, and in this species expansion seems to be the characteristic reaction of both fore and hind limbs to acetyl-choline. With histamine and depressor doses of adrenaline, on the other hand, the hind leg with normal nervous connexions shows any kind of volume response from pure expansion to pure constriction. It may be noted, in passing, that evidence of the same variability in the volume change of the limbs may be found in a number of publications dealing with the action of the "depressor substances" found in various organ extracts and products of protein hydrolysis, of which, as Dale and Laidlaw pointed out, the action on the blood vessels belongs to the same general type as that of histamine. Some authors have regarded such differences as due to specific activities of the extracts from different organs on particular parts of the vascular system (13). Thus one extract will be described as causing vasodilatation in the intestine but constriction in the leg, while another is reputed to cause a greater dilatation in the leg than in the intestine. In the case of some organ-extracts the effect is probably complicated by admixture of pressor with the predominant depressor substances; but the fact that we observe quite as wide a variation in the volume changes of the leg in a series of cats as a result of injecting into each the same dose (0·01–0·02 mgm.) of the pure substance histamine, makes it impossible to accept differences of this kind as indicating a specific localisation of the effects of organ-extracts. Between simple expansion and simple shrinkage all varieties of mixed effect can be observed in the legs of different cats with small intravenous doses of histamine—a small preliminary expansion followed by a more pronounced constriction outlasting the fall of blood-pressure, an expansion interrupted midway by a constriction, and so on.

Several of these varieties can be seen in records reproduced in this paper.

The volume change of the normally innervated leg of the cat, accompanying the fall of blood-pressure usually produced in this species under ether anæsthesia by a small dose of adrenaline (0·001–0·005 mgm.), not only shows the same range of variation as that produced by small doses of histamine, but in practically every instance the particular form of the plethysmogram produced by the leg of a particular cat in response to histamine is reproduced with suggestive fidelity in response to adrenaline. Cf. Figs. 5, 9 (left leg), and 11 (left leg). Such difference as can be detected between the volume changes in the leg of any one cat in response to these two substances is practically always in one direction—a greater prominence of the constrictor phase of the action in the case of adrenaline than in the case of histamine.

This tendency of the constrictor action to greater relative prominence with adrenaline gives a clear hint as to its nature. It cannot be due to the excitation of the vasomotor centre, which is stated to follow a fall of general arterial pressure as a result of bulbar anæmia (cf. Hill(14)), since, if it were so, it should be most prominent in the case of histamine and acetyl-choline, which in the doses usually employed by us cause a much larger fall of arterial pressure than the greatest obtainable with adrenaline; nor is it possible to attribute to such secondary action of the vasomotor centre a constrictor action which sometimes begins as soon as the fall of pressure. In the same way it cannot be mainly a mere passive effect of the general fall of pressure, for in this case again it should be proportionate to the fall produced, and should not outlast the depressor effect, as it frequently does. The nature of this constrictor phase of the action of these two substances, and the consequent complexity and variability of the plethysmograms given especially by the hind leg in response to them, becomes clearer when it is remembered that each of them possesses not only the vasodilator action which is the subject of our immediate study, but a vasoconstrictor action. This, in the case of adrenaline, is the more obvious and characteristic effect, becoming generally predominant when the dosage is but slightly raised beyond about 0·005 mgm., which is the maximum dose with which, in the average cat under ether, a purely depressor effect with adrenaline is to be expected. Any untoward influence, such as undue prolongation of the experiment, even though attended with but a small decline of level of the arterial pressure, usually results in the replacement of the depressor by a pressor action of adrenaline even in this lower range of

dosage. In the case of histamine the general vasodilator fall of pressure is a more stable and characteristic effect of small doses, and the fact that these also possess a vasoconstrictor action only becomes distinct under conditions of artificial perfusion; but experiments of the latter kind leave no doubt as to its existence. Acetyl-choline, on the other hand, appears to have a purely vasodilator action in the low range of dosage which we have used throughout these experiments. The vasoconstrictor effects of relatively very large doses have been discussed elsewhere by Dale and by Hunt and do not concern us here. A primary difference between the effects of these substances in the doses with which we are dealing is, therefore, that with either histamine or adrenaline we have a complex of opposed actions, a vasodilator and a vasoconstrictor effect, either of which may in a particular case be predominant in an organ such as the leg, whereas small doses of acetyl-choline have a purely vasodilator action. The relative uncertainty of the volume-effect with histamine and adrenaline and constancy of that with acetyl-choline find in this difference a sufficient explanation. The only point needing additional emphasis at this stage is the aforementioned parallelism between the effects of histamine and adrenaline on the leg volume in different experiments. In both cases we now know that both types of effect, dilator and constrictor, are due to direct and peripheral action; so that the parallelism between the effects of the two substances can only be interpreted as indicating that the conditions of vascular tone which favour the predominance of either vasoconstriction or vasodilatation in the normally innervated leg are the same for histamine as for adrenaline, while the vasodilator action of acetyl-choline is relatively independent of such conditions.

The explanation which immediately suggests itself is that which Cannon and Lyman(15) gave for the variations in the effect of small doses of adrenaline on the cat's blood-pressure, namely, that the condition determining the response in the direction of vasodilatation or vasoconstriction is simply the state of arterial tone at the time of the experiment. On this view, when the tone of arteries in the leg is high histamine or adrenaline should cause them to relax, when it is low to constrict, so that in either case the tone approaches what Cannon and Lyman call a "critical level." The sufficiency of this simple conception can be tested by observing how the volume-response of the leg to these substances is affected by changes in arterial tone deliberately introduced. So long as it leaves unimpaired the vitality and sensitiveness of arterial

muscle, any influence which produces relaxation of the arteries in the leg should tend to promotion of the constrictor and elimination of the

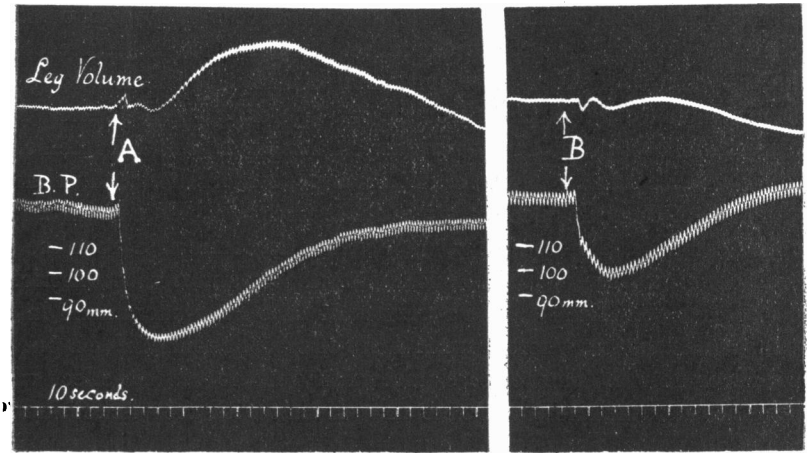

Fig. 5. Volume of normal leg and blood-pressure. At *A* 0·02 mgm. histamine; at *B* 0·004 mgm. adrenaline.

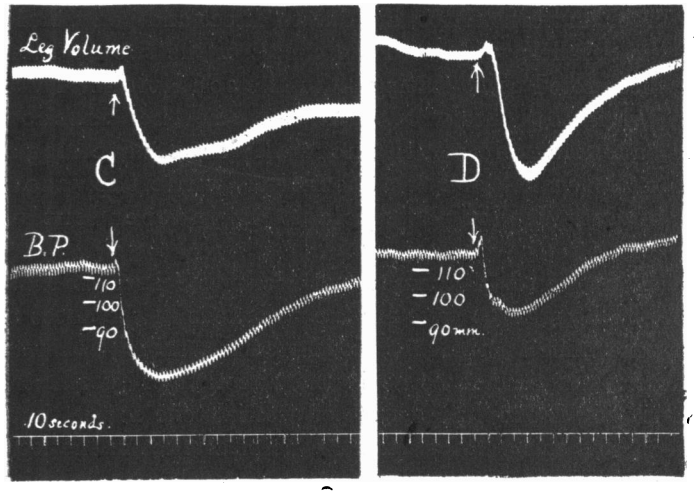

Fig. 6. Continuation of experiment shown in Fig. 5. Sciatic and anterior crural nerves have now been cut. At *C* and *D* 0·02 mgm. of histamine and 0·004 mgm. of adrenaline, as before.

dilator action of both these substances. We shall see that under this test the explanation fails.

(iii) *Immediate effects of nerve section.* When the sciatic and anterior crural nerves are cut the immediate effect is a dilatation of the arteries of the leg, which, as Bayliss(16) has shown, is not adequately explained by the interruption of the flow of impulses from the vasomotor centre along sympathetic fibres, but is due in part to the excitation of sensory vasodilators by the mechanical stimulus of the section. The dilatation is immediately made obvious in the plethysmogram by the large increase in the amplitude of the volume-pulse. The persistence of the effect is variable, but the excess of volume-pulse in the decentralised limb usually remains obvious throughout the further course of an ordinary experiment.

In cases in which histamine and adrenaline cause a dilatation of the limb while the nerves are intact, it sometimes happens that the same doses, injected again immediately after the nerves are cut, cause a simple shrinkage of the limb, which may have the appearance of passively following the fall of arterial pressure. Such a change of response is illustrated in Figs. 5 and 6 which further illustrate what was said above concerning the greater relative prominence of the constrictor element in the action of adrenaline. Hartmann and Fraser's contention that the vasodilator action of adrenaline is central in origin was partly based on observations of this kind. This, however, is by no means a constant or even the most frequent and characteristic type of change in response to these substances produced by section of the nerves. Figs 5 and 6, indeed, are taken from the only experiment, among many which we made, in which the change in this direction was thus definite. Fig. 7 shows an entirely different type of change, the practically pure constrictor response of the normal (left) leg to 0·01 mgm. of histamine being replaced by simple dilatation in the case of the other (right) leg, the nerves to which have

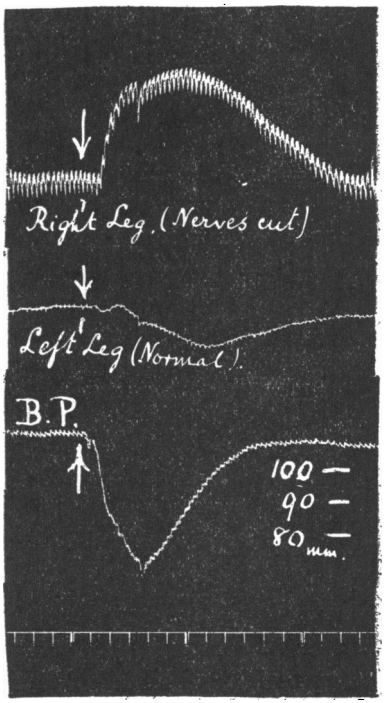

Fig. 7. Effect of 0·01 mgm. histamine. Volumes of leg with nerves freshly cut and of normal leg; blood-pressure.

been cut a few minutes previously. Note the greatly exaggerated volume-pulse in the right leg, indicating that the arterial dilatation produced by nerve section still persists. It may be added, as of incidental interest, that the cat giving this record showed a very high degree of sensitiveness to histamine. Tests for the lower limit of effective dosage showed a distinct fall of arterial blood-pressure and definite expansion of the right (decentralised) leg with 0·000,000,01 mgm., but no effect with one-tenth of this dose. The limit was therefore the same as that found by Hunt for acetyl-choline. Such extreme sensitiveness to histamine is, however, not usual. Fig. 8 is taken from the record of an experiment on a cat in which the nerves to the left leg had been cut 26 days

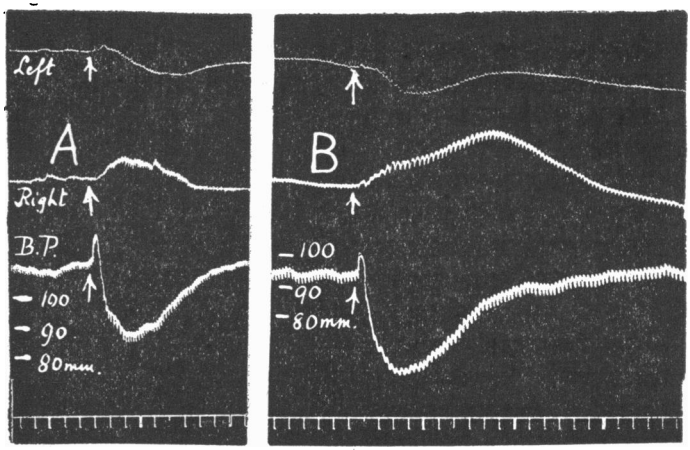

Fig. 8. Left leg completely denervated (unusual constrictor response); nerves to right leg freshly cut. At *A* 0·001 mgm. adrenaline; at *B* 0·01 mgm. histamine.

previously. At the beginning of the experiment injections of small doses of adrenaline and histamine (in each case 0·01 mgm.) caused dilatation of the left (denervated) and constriction of the right (normally innervated) leg. We shall see later that this readier response of the denervated leg to the dilator action is the general rule. In the middle of the experiment the nerves to the right leg were cut, and the same doses of adrenaline and histamine repeated. Although the arteries become somewhat dilated in consequence, as shown by the increased volume-pulse, 0·001 mgm. of adrenaline, at *A*, now causes distinct expansion of the right leg, subjected to recent nerve section, while the completely denervated left leg now constricts. This constriction of the denervated limb is a very uncommon result in our experience, and it is the more interesting, therefore, to

observe that the same reversal of reaction has occurred during the experiment in the case of histamine, which now (at *B*) similarly causes shrinkage of the completely denervated left leg, expansion of the recently decentralised right.

In a series of experiments on the immediate changes in response due to nerve section this correspondence between the effect of histamine and adrenaline is clearly traceable. It can be said with certainty that if a small dose of histamine (of the order of 0·01 mgm.) causes constriction of a limb, adrenaline in similar low dosage also causes constriction; if adrenaline causes dilatation histamine causes dilatation. The converse statements do not always hold good, for, as already stated, the dilator effect of adrenaline is more liable to be overpowered by its constrictor effect, so that cases may be found in which histamine causes dilatation while adrenaline causes constriction. But the broad agreement is sufficiently striking to warrant the suggestion that there is some condition of the limb vessels which favours in common the vasodilator effects of histamine and of adrenaline, and that this condition is not the state of tone of the arteries. We shall have to consider also the effect of changes in arterial tone on the vasoconstrictor action, which complicates the vasodilator action even of small doses of histamine and adrenaline, and in the case of the latter becomes the predominant effect when the dose is raised above a certain low level. Before doing so, however, it will be convenient to examine the immediate effect of nerve section on the response to acetyl-choline, which has apparently an unmixed vasodilator action in the doses with which we are concerned. We have made fewer such experiments with this substance than with the others, but the results have been uniform and have shown a very significant divergence from those obtained with histamine and adrenaline, when the changes of response to all three were recorded in the same experiment. When the nerves to the leg in the plethysmograph were cut, and the arteries in consequence became dilated, the expansion shown by the leg in response to a small dose (*e.g.* 0·001 mgm.) of acetyl-choline has always been conspicuously less than that shown by the same leg while its nerves were intact, even when the effect of the nerve section was so to increase the dilator action of histamine and adrenaline that a constrictor action of these substances on the normal limb was replaced by a dilator action when the nerves were cut. In the experiment of which Fig. 9 reproduces part of the record, both limbs had constricted while their nerves were intact in response to small doses of histamine (0·01 mgm.) and adrenaline (0·004 mgm.). Under the same

condition the two limbs had shown approximately equal expansions in response to acetyl-choline (0·001 mgm.). The nerves (sciatic and anterior crural) of the right leg were then cut, and the doses repeated, with the results shown in Fig. 9. It will be seen that the intact left leg still shrinks in response to histamine and adrenaline, but dilates well with acetyl-choline. The effect of nerve section on the right leg, the arterial dilatation in which is, as usual, visible in the large volume-pulse, is to cause this limb to show a small, but quite definite expansion with adrenaline and histamine, whereas its expansion with acetyl-choline is very small in comparison with that of the normal leg. Fig. 10 shows a similar comparison of the volume effects of histamine and acetyl-choline

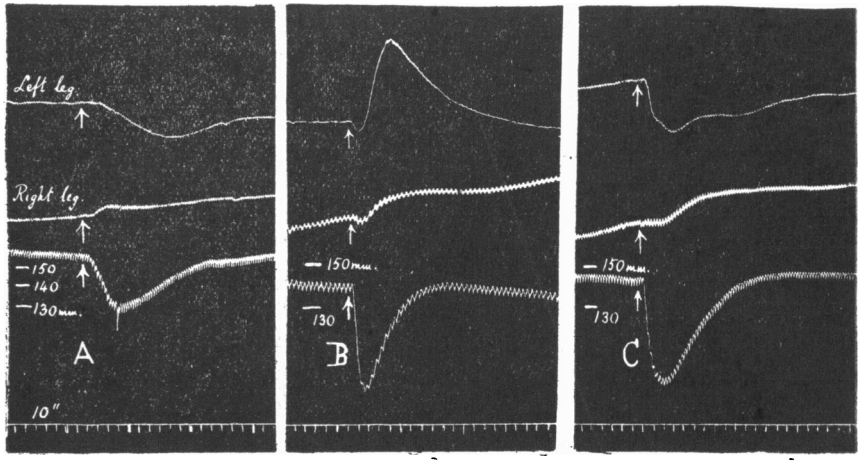

Fig. 9. Left leg normal, nerves to right leg freshly cut. At *A*, 0·004 mgm. adrenaline; at *B*, 0·001 mgm. acetyl-choline; at *C*, 0·01 mgm. histamine.

on a normal (left) leg and freshly decentralised (right) leg. Again it will be seen that the cutting of the nerves, with its resultant arterial dilatation, has caused an increase in the dilating action of histamine, a decrease in that of acetyl-choline.

Such a result, considered by itself, might be taken to indicate that the vasodilator action of acetyl-choline was partly produced through action on nerve centres. This interpretation is excluded, however, by the effect of allowing the nerves to degenerate completely after section, when, as will be described below, the dilatation in response to acetyl-choline becomes greater than that of the normal limb. The diminished reaction immediately following nerve section must, therefore, be related

to the change in tone of the limb vessels which is the immediate consequence of the operation. The facts are entirely compatible with the view that the dilator action of acetyl-choline is due to inhibition of the tone of arterial muscle; when this tone is already relaxed as the result of the vasodilator stimulus of nerve section acetyl-choline produces a smaller dilatation.

It is beyond doubt, also, that the vasoconstrictor actions of histamine and adrenaline are, at any rate, largely effects on arterial muscle. That contraction of isolated strips, from arteries of different parts of the

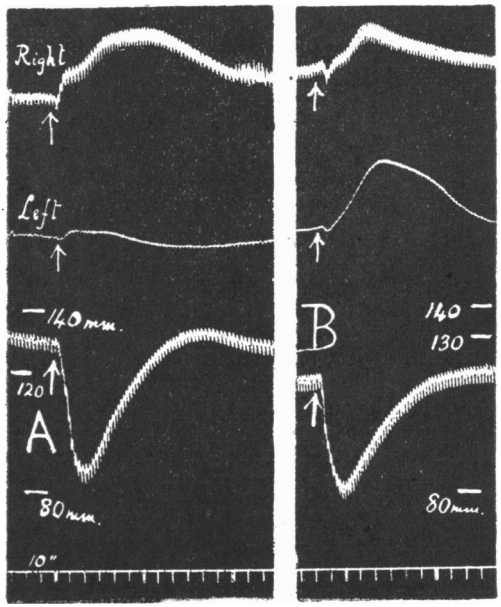

Fig. 10. Left leg normal, nerves to right leg freshly cut. At *A*, 0·01 mgm. histamine; at *B*, 0·001 mgm. acetyl-choline.

system, is produced by minute doses of both these substances has been shown by numerous workers on their action. Later in this paper we shall produce evidence of the constriction produced by histamine on a purely arterial system perfused artificially. We should expect, accordingly, that relaxation of the arteries of an organ as caused by recent section of the nerves would intensify the constrictor action of these substances by giving a wider range for its action. The point is obviously difficult of demonstration by ordinary plethysmographic methods, for, as we have seen, the vasodilator action of very small doses of these

substances is more often intensified than impaired by such nerve section, so that the impression is often obtained that the constrictor effect has been weakened by the arterial dilatation following nerve section. This impression can usually be corrected by pushing the dose higher, when the superiority of the constrictor effect also in the decentralised limb becomes obvious.

Occasionally, in common with others who have worked on the subject, we have come across a cat in which even the smallest doses of adrenaline produced practically no vasodilatation, though histamine acted as usual; and in such cases we have observed an intensification of the constrictor action of small doses of adrenaline as the result of nerve section, though the dilator effect of histamine was increased by the same procedure. So far, then, as the complexity of the response to histamine and adrenaline allows us to determine it, it would appear that, in the constrictor effect of both these substances, as in the dilator effect of acetyl-choline, we have probably an action on arterial muscle, varying in the range of its effect with the state of tone of the arteries. In the case of adrenaline this is, of course, the obvious and prevalent conception of its vasoconstrictor effect. We have seen, on the other hand, that their dilator effect varies in a manner having no apparent relation to arterial tone and the question necessarily arises whether there is any other structure in the vascular mechanism, capable of maintaining a tone which, like that of the arteries, relaxes in response to an appropriate stimulus, and which varies independently of the tone of the arterial muscle. The suggestion almost inevitably presents itself, that the dilator effect may be due to action on the walls of the capillaries. We may leave it as a mere suggestion at present, until we have considered evidence of other kinds.

(iv) *Effect of complete denervation.* We have already seen that the leg to which the nerves have been allowed to degenerate completely after section gives a full dilator response to small doses of all the three substances with which we are dealing. We have now to add that this dilator effect is in all three cases habitually, though not quite invariably, much larger than that shown by the corresponding normally innervated limb. Fig. 8 shows an exceptional case of constrictor reaction to small doses of both adrenaline (*A*) and histamine (*B*) after complete denervation (left leg). There is, indeed, a general increase of responsiveness to chemical stimuli of all kinds; thus in one case the denervated limb showed a much larger dilatation than the normal, not only with the three substances specially under consideration, but with amyl nitrite

and caffeine as well. Such a sensitized limb further showed a much more pronounced constriction than the normal with a pressor dose (*e.g.* 0·05 mgm.) of adrenaline. Possibly this concomitant increase of sensitiveness to vasoconstrictor effects affords an explanation of the rare cases in which we observed that a moderate dose of histamine (0·02 mgm.) caused smaller expansion of a denervated limb than of the corresponding normal, which happened in such cases to expand unusually well.

The experiments on the denervated leg with acetyl-choline and the other more normal vasodilator substances, such as amyl nitrite, have only been made hitherto after an interval sufficient to allow complete degeneration (14 days and upwards after section). With histamine, on the other hand, and in a few cases with adrenaline also, we have seen that the improved dilator response appears almost immediately after the nerves have been divided, at a time when the dilator response to acetyl-choline is weakened by the slackness of the arterial tone. We have observed the exaggerated dilator response to histamine at all periods from 15 minutes to 2 months after nerve section. It occurred to us that observation of the state of circulation in the denervated as contrasted with that in the normal leg might throw further light on the mode of action.

The flushing of a limb as the immediate result of section of its nerves has been known since the early experiments of Bernard and of Schiff. There is no clear and consistent account, however, of the relation of the temperature of the limb to the state of tone of its superficial vessels, as judged by inspection, during and after degeneration of the nerves following section. Goltz(17) observed that the dog's limb with cut nerves remained warmer than the normal limb for varying periods up to four weeks after the operation. Luchsinger(18), using cats with unpigmented feet, described the pads of the operated foot as redder than those of the normal for 3 to 4 days after section, and thereafter as of equal redness at the ordinary temperature, though at any period the normal pads became the redder if the animal was warmed. We have observed a persistent and curious form of contrast which does not seem to have been noticed previously. We also used cats with unpigmented feet. In our experience the flushing which follows immediately on section of the sciatic and anterior crural nerves passes off rapidly. Often by the time the small operation wound has been sewn up, and while the animal is fully under the influence of the anæsthetic, the operated foot has become distinctly paler than the normal control. On the following day, and for

the rest of the period of observation (up to 2 months), the contrast presented by the two feet is striking and suggestive, the pads of the

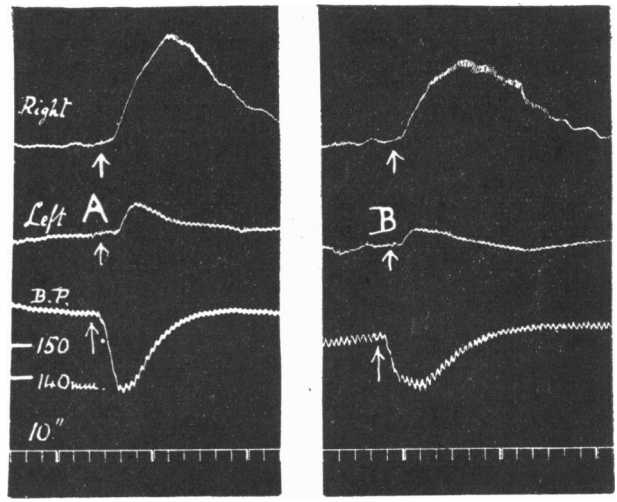

Fig. 11. Right sciatic and anterior crural nerves cut 2 days previously; left leg normal. At *A*, 0·001 mgm. histamine; at *B*, 0·001 mgm. adrenaline.

denervated leg being as a rule conspicuously *paler* than the normal, and at the same time very noticeably and surprisingly *warmer* to the touch. The contrast is especially conspicuous in cold weather, under which conditions the pads of the normal hind foot are cold to the touch, though they usually present a bright, slightly bluish pink colour. In cases in which this contrast between warm pallor and cold congestion has been well marked, we have invariably observed a conspicuously superior dilator response to histamine on the part of the denervated leg, often contrasting with a constriction of the normal. Fig. 11 shows the response of a leg two days after section of the nerves, and of the corresponding normal leg, to 0·001 mgm. doses of histamine (*A*) and adrenaline (*B*). The similarity of the two effects is clearly shown. Fig. 12 shows the reaction to 0·01 mgm. of histamine 5 days after nerve section, in a cat in which this contrast of temperature and colour was consistently well marked.

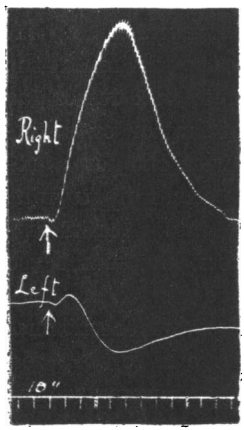

Fig. 12. Right sciatic and anterior crural nerves cut 5 days previously. Left leg normal. Effect of 0·01 mgm. histamine.

It seemed highly improbable that the difference in surface temperature was due to defect of evaporative cooling on the operated side, owing to the paralysis of the sweat glands on the pads, and a simple experiment established the reality of the difference and its independence of sweating. We compared the rates at which the two paws raised the temperature of equal quantities of cold water, in which they were immersed. The cat used was that which furnished the record reproduced in Fig. 12. Into each of two small glass cylinders, fitting easily over the paws, 10 c.c. of water, at room temperature, were poured. The paws were immersed to equal depths and a thermometer was inserted into the water alongside each. Slight up-and-down movements of the cylinders kept the water well mixed. The following figures show the rates at which the temperature rose in the two cases:

Time after immersion in seconds	Temperature of water	
	Denervated paw	Normal paw
	°	°
0	11	11
45	15	13·75
60	17	14
90	18	14·5
120	18·5	15
150	18·5	15·5
180	18·5	16
210	19	17
230	19·5	17·5
250	19	18
280	19	18·5
300	19	19
345	20	19
405	21	20
450	22	20·5

It will be seen that the greatest difference in rate of warming occurs soon after immersion, the maximum difference between the two temperatures being reached at 1½ minutes. Such a difference in the rates at which the paws give up heat to water surrounding them can only have one explanation. It is not credible that the blood reaching the skin by the arteries in the two cases differs significantly in temperature. The only reasonable explanation is that the blood is circulating more rapidly through the denervated paw than through the normal, and this greater rapidity of circulation, associated with surface-pallor, can only be attributed to the capillary area being more restricted in relation to the volume of blood reaching it by the arteries. In other words, the relative pallor and warmth of the denervated paw must be due, not to constriction of

the arteries supplying it, but to a condition of tone of the capillaries, such that the blood courses through them in a restricted but rapid current; the relative flush and coldness of the normal paw must be due not to a more dilated condition of its arteries, but to such relative width of its capillaries that the blood spreads out in them into a broad, sluggish stream. The difference may be compared with that seen between the hands of different persons in cold weather. In one, with good tone and reactivity of the capillaries, the skin of the hands is pale but warm; in another, in whom the capillaries are atonic and poorly reactive, the hands are dusky red, congested and unpleasantly cold to the touch, owing to the sluggish circulation through the dilated capillaries.

It is of interest to note that a smaller difference in surface temperature can often be observed between the fore-paws and hind-paws of a cat in cold weather, the fore-paws being distinctly warmer to the touch. In such cases we have observed a similar contrast in the reactions to histamine, the fore-limb dilating with a dose which causes constriction of the hind-limb (Fig. 13).

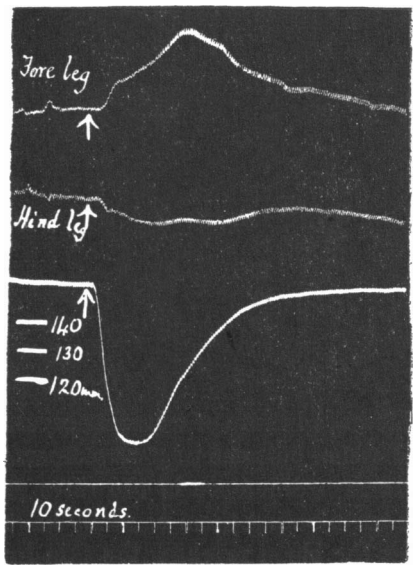

Fig. 13. Effect of 0·04 mgm. histamine on volumes of normal fore and hind legs and blood-pressure.

If observation were limited to the condition after complete degeneration, the association of this condition, which we regard as indicating intensified reactivity of the capillaries, with enhancement of the dilator reaction to histamine and adrenaline, could not be regarded as of great significance; for at this stage the reaction is also abnormally sensitive to acetyl-choline, amyl nitrite, etc., the action of which on arterial muscle is not to be doubted. We have shown, however, in dealing with the immediate results of nerve section, that there is frequently recognisable a stage at which, though the arterial dilatation resulting from nerve section still persists, the dilator reaction to histamine and adrenaline is already intensified while that to acetyl-choline is diminished. It was of interest, therefore, to observe the state of capil-

lary tone at this stage. The following experimental record deals with observations made without the aid of the plethysmograph, the unpigmented pads of the hind feet being merely inspected.

In a large male cat, with unpigmented feet, the sciatic and anterior crural nerves on the right side were cut aseptically under ether anæsthesia. As soon as the small wounds had been stitched and dressed the hind feet were inspected. The pads on the right (operated) side were found to be distinctly redder than those on the left. The right pads were also slightly the warmer to the touch, and were sweating profusely. They rapidly grew paler and the sweating ceased. Five minutes later the right pads were conspicuously paler than the normal left pads, but now felt definitely warmer. They were watched a few minutes longer, to make sure that this contrast had become stable. Intravenous injections of small doses of histamine, acetyl-choline and adrenaline were then given, the cat being still under the influence of ether. After each injection the stable contrast was re-established before the next was given, so that at the time of giving each injection the right pads were paler than the left.

Histamine 0·01 mgm. Right pads flush, while left become slightly paler. While the flush on the right side is passing off a weaker flush on the left side appears, outlasting the more pronounced effect on the right. Both sides then became paler, and the original contrast is slowly restored and becomes stable.

Acetyl-choline 0·001 mgm. Right pads show no definite change of colour; left pads show a well-marked flush, the contrast with the right being decidedly intensified. This passes off and the original condition is restored.

These doses were then repeated, and another observer, unaware of the order in which the drugs were being injected, reported the same effects.

Adrenaline 0·001 mgm. After a longer latent period than that seen with the other two, the right pads show a pronounced flush, while the left become paler so that the right become decidedly the redder. As the effect on the right pads subsides a weaker secondary flush is seen on the left side. This passes off leaving both sides pale, and the original contrast is then slowly re-established.

It will be seen that, in this case, the condition of pallor and relative warmth has already appeared as the result of nerve section, and histamine and adrenaline already show an enhanced dilator effect on the operated side, at a period 10 to 20 minutes after the nerves had been cut. The uniform result of our plethysmographic records has been to show that at this period the arteries are still dilated, the volume-pulse being much increased on the operated side. Whenever it has been tried the dilator effect of acetyl-choline has been diminished at the corresponding stage. Similarly in these observations acetyl-choline produced no perceptible reddening of the pads on the operated side, though it caused distinct flushing of the normal pads.

For our present purpose the important points are that the condition of relative pallor and warmth may appear very early after nerve section, and with it an exaggerated dilator response to small doses of histamine and adrenaline, at a stage when the dilator response to acetyl-choline

is depressed. In so far then as we are correct in interpreting the warm pallor as indicative of capillary tone, the suggestion is strengthened that histamine and adrenaline exert their dilator action on capillary walls, while acetyl-choline dilates arterioles.

One recent experiment should be mentioned, in which the nerves of the leg were thoroughly cocainised before section. In this instance the dilator action of acetyl-choline also was increased as the immediate result of the nerve-section. It seems clear, then, that the loss of arterial tone which normally reduces the effect of acetyl-choline at this stage is due to stimulation of vasodilator fibres, and not merely to loss of constrictor tone.

(v) *Effects of prolonged anæmia and cooling.* In searching for an explanation of the failure of histamine to exhibit its vasodilator action on the artificially perfused vessels of a surviving organ, we considered the possibility that the interval between cessation of the normal and commencement of the artificial circulation might play a part. We shall later show reason for believing this suggestion to be incorrect, but it led us to test the influence on the response to histamine of temporary anæmia produced by clamping the arteries to an organ in the living animal. Later we made comparative tests of the alteration in the reaction to acetyl-choline produced by the same procedure. It proved impracticable to test the effect of such treatment on the vasodilator effect of adrenaline. We have already drawn attention to the relative impermanence of this effect, owing to the fact that it readily becomes overpowered by the vasoconstrictor action, even with small doses. In several attempts to determine the effect of temporary anæmia we observed, indeed, that a vasodilator was replaced by a vasoconstrictor effect of adrenaline in the organ under experiment; since, however, the action on the general arterial pressure had simultaneously changed from a depressor to a pressor effect, the local result could not be regarded as significant. We confined our attention, therefore, to the changes in the action of histamine and acetyl-choline under these conditions. A few experiments were made on normally innervated legs showing a good dilatation with histamine, but for most of these experiments on the effects of temporary anæmia we chose cats in which the nerves to one hind leg had been allowed to degenerate after section. We had, therefore, an organ which could be depended on to give initially a full dilator response to both histamine and acetyl-choline. Precautions were necessary to guard against an anastomotic supply of blood to the limb while the vessels were occluded, so that a real ischæmia could be established.

For this purpose all branches from the lower end of the aorta and vena cava to the lumbar muscles, etc., were tied up to about 1 cm. above the origin of the inferior mesenteric artery. The latter was also tied, cut, and the lower end of the rectum double ligatured and cut through. In female cats the vagina and the vessels accompanying it were similarly double ligatured and divided and the ovarian vessels tied off. The aorta was tied between the origin of the external iliac arteries and its terminal bifurcation into the internal iliacs, which have this separate origin in the cat. The muscles and skin of the back and abdominal wall, at a level just above the origin of the inferior mesenteric artery, were then mass ligatured in three sections, the strong string ligatures being passed by transfixion.

The external iliac artery and vein on the side of the normal leg were clamped close to their junction with aorta and vena cava and ligatured about a centimetre lower down. A cannula was inserted into each between ligature and clamp. Through these the vessels of the denervated limb could be washed out when the aorta and vena cava were clamped. Cannulæ for recording blood-pressure and making injections having been tied into a carotid artery and jugular vein, a plethysmograph was applied to the denervated leg, which was to be the subject of observation, and the record started. Preliminary injections of histamine and acetyl-choline were first given. The vasodilator effects of two substances on such a denervated limb give curves of very similar contour, but when doses are chosen which cause about equal falls of general arterial pressure the expansion of the leg caused by histamine is as a rule distinctly the greater.

When the vessels to the leg are occluded, even for a brief period, and the circulation then restored by their release, the immediate effect is a wide dilatation of the vessels of the leg, as shown by the great increase in volume of the limb beyond its original size and the very large volume-pulse. Roy and Graham Brown(19) have shown that this dilatation after temporary anæmia affects not only the tone of the arterioles but of the capillaries also. At this stage, if a record is taken as soon as the volume of the limb has become approximately stable, the fall of arterial pressure caused by injection of either histamine or acetyl-choline is accompanied by a shrinkage of the limb which has all the appearance of a merely passive effect. As the vessels regain tone the dilator effect of acetyl-choline reappears and later that of histamine also. The dilator effect of histamine, however, recovers at a distinctly lower rate than that of acetyl-choline. Nevertheless it eventually reappeared, even after the flow of blood through the leg had been stopped for as long as an hour. The disparity between the rates of recovery of the action of the two substances was apparently accentuated if the anæmia was rendered more complete, and the additional factor of cooling during the anæmic period

introduced, by washing the vessels of the leg out with cold saline, as above described, and temporarily replacing the warm water in the plethysmograph with cold. When the normal circulation was again restored a stage of recovery could be found at which acetyl-choline produced a definite dilator action, while a dose of histamine, causing an approximately equal fall of arterial pressure, produced a simple constriction of the limb (Fig. 14). Later, when the reaction to acetyl-choline had still further recovered, histamine again caused dilatation of the limb, but the effect was relatively a small one, whereas, when the same doses of the two substances were given before the period of anæmia and cooling, the dilator effect of histamine was distinctly the greater.

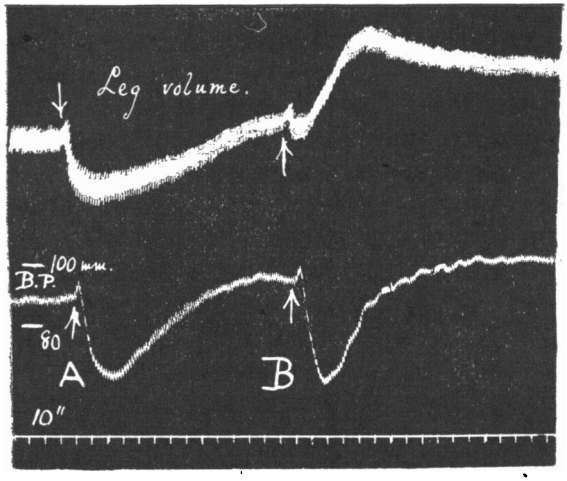

Fig. 14. Volume of leg after anæmia and cooling; blood-pressure. At *A*, 0·01 mgm. histamine; at *B*, 0·001 mgm. acetyl-choline.

Comparing the effects of temporary anæmia with those immediately following nerve section, we have in both cases a long persistent dilatation of the vessels of the limb. This causes, in both instances, a weakening of the dilator effect of acetyl-choline, in accordance with the expectation that an arterial dilator will exhibit relatively little action on arteries already dilated. In the case of histamine, on the other hand, we have the significant contrast, that whereas its dilator effect may be and indeed usually is accentuated during the period of dilatation following nerve section, in that following anæmia its dilator effect is weakened to a greater degree and recovered more slowly than that of acetyl-choline. Again the evidence suggests that the vasodilator action of histamine is

produced on some part of the system of blood vessels other than the plain muscle of the arterioles, and the additional conclusion is indicated that the structure in question is one of which the tone is not necessarily weakened by the stimulus of fresh nerve section, but is more seriously and lastingly impaired by anæmia and cooling than that of the arterial muscle. The evidence, so far as it goes, is again consistent with the view that the dilator action of histamine is an effect on the tone of the capillaries; for there is no evidence that this tone is regularly weakened by nerve section like that of the arteries, while there is evidence of its susceptibility to temporary anæmia and cold.

IV. Distribution of the vasodilator action.

Before we consider the distribution of the effects in the different organs of one animal, there are points of interest to be noted in the manner in which the vasodilator action of the three substances appears in different species. The vasodilator action of acetyl-choline has been observed in all animals on which the experiment has been made. Hunt quotes experiments on rabbits, cats and dogs. We have ourselves observed the effect in a monkey as well. There is no apparent difference in the order of intensity of the effect in the different types.

The vasodilator effect of adrenaline, on the other hand, has hitherto only been described in carnivora. It seems definitely to be absent in the rodents. It is at least highly suggestive that the vasodilator action of histamine shows an analogous specific restriction. In the cat and dog it is the obvious and predominant feature of the effect on the circulation; in these species a vasoconstrictor action is also present, but only becomes obvious in special circumstances. In the rabbit and guinea-pig, on the other hand, Dale and Laidlaw found that the effect of histamine, under conditions excluding complications due to interference with respiration, was a simple rise of blood-pressure due to vasoconstriction. In some recent experiments on the effect of histamine on anæsthetised rats we found no evidence of the depressor, vasodilator action, though this species has provided the hitherto unique example of plain muscle (uterus) of which the tone and rhythm are inhibited by histamine (Guggenheim). It is not known how far the correspondence in distribution between the vasodilator actions of histamine and adrenaline holds good for other species. Dale and Laidlaw observed a very pronounced vasodilator action of histamine in the monkey, and a depressor action, probably of the same type, in the fowl, but the reaction of these

types with minute doses of adrenaline has apparently not been recorded. We have not as yet had the opportunity of observing it ourselves. The persistent pressor action of larger doses of adrenaline, observed by Barger and Dale(20) after ergotoxine, does not necessarily show that adrenaline has no vasodilator action in this species, but only that the vasoconstrictor action is not eliminated by ergotoxine. So far, then, the action of all three substances has only been compared in the cat, dog, and rodents; and of these only the cat and dog show vasodilator effects with adrenaline or histamine, while acetyl-choline, like better known vasodilators such as the nitrites, shows similar effects in all. The vasoconstrictor actions of adrenaline and histamine, again, can be detected in carnivora and rodents alike.

In comparing the action of the different substances on different organs and tissues we are less inclined than some writers to attribute importance to quantitative differences, since experience has convinced us how widely the volume-change exhibited by any organ may vary with changes in the tone and responsiveness of its vessels. Certain broad and apparently constant differences of reaction may be noted. The kidney seems peculiarly insensitive to the vasodilator action of all three substances, showing a reduction in volume when other organs expand. Here again, however, a difference may be observed, in that with acetyl-choline the shrinkage seems to be purely a passive effect of the general fall of blood-pressure, while histamine and adrenaline, in doses producing a depression of the same magnitude, cause a shrinkage of the kidney disproportionately great. The circulatory scheme of the kidney, however, with its secondary system of efferent arterioles from the glomeruli, presents complexities which make interpretation of its volume changes peculiarly difficult.

The point probably of most interest is the distribution of the dilator effect between the vessels of the skin and those of the skeletal muscles. Hoskins, Gunning and Berry(21) have drawn attention to the greater regularity and extent of the dilator effect of adrenaline in the bared muscles of a limb than in the whole limb with its normal covering of skin. We have observed a parallel contrast in the case of histamine. When a cat's leg constricted with histamine and with depressor doses of adrenaline, we found, on removing the skin and amputating the foot at the ankle, and replacing the warm water in the plethysmograph with saline at the same temperature, that the bared musculature of the leg showed expansion with the same doses of both substances (Fig. 15). If we can assume that the response of the vessels in the musculature has not been

altered by the operation of skinning, it is evident that the preponderant constriction seen with the intact leg must have been an effect on skin vessels. We do not regard this, however, as necessarily indicating a specific difference of reaction between the vessels of the skin and those of the muscles. When the leg has been denervated, by allowing time for degeneration of its nerves after section, there can be no doubt that the skin vessels participate in the large expansion of the whole leg in response to histamine or adrenaline. In unpigmented pads of the feet the vasodilator effect can then be directly observed, and in a leg so sensitized to vasodilator effects we found that removal of the skin merely reduced the extent of the expansion in response to histamine. (The effect of skinning the denervated leg on its response to adrenaline was unfortunately not tested.) We are disposed, therefore, to regard the predominance of constrictor effects frequently seen in the normally innervated limb, and the increased prominence of the dilator effects produced in such a limb by removing the skin, as indicating that the normal vasomotor control, affecting chiefly the skin vessels, tends to produce the conditions of tonus which we have described in an earlier section as favouring the preponderance of the constrictor over the dilator effects of histamine and adrenaline. As we there pointed out, this condition is especially liable to be found in the hind limbs during cold weather, and it is obvious that the vasomotor adjustment to external temperature will affect the vessels of the skin especially.

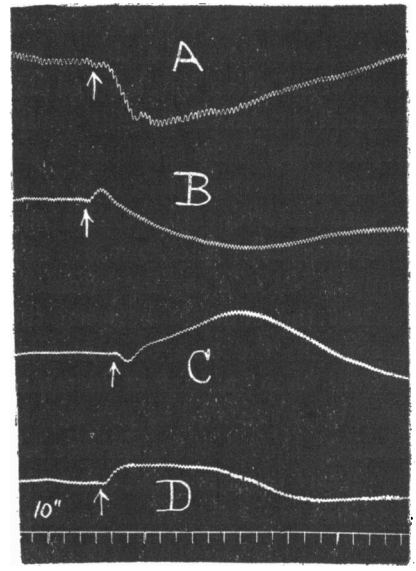

Fig. 15. Effect of skinning the leg on volume-response to histamine and adrenaline.

A—0·02 mgm. histamine } on intact
B—0·002 mgm. adrenaline } leg.
C—0·02 mgm. histamine } on skinned
D—0·004 mgm. adrenaline } leg.

The meaning of the varying reaction of the skin vessels to histamine and adrenaline, and of the relatively constant dilator response of those of the muscles, becomes clearer in the light of the evidence which we have already given, and which we shall later reinforce by more direct evidence,

in support of the suggestion that the vasodilator action of these substances is an effect on capillaries, the antagonistic vasoconstrictor action an effect mainly on arterial muscle. The variable tone of the arterioles of the skin, and its susceptibility to sympathetic control, are familiar and conspicuous, while the effect of nervous impulses on the arterioles to skeletal muscles is obscure and uncertain, being complicated under conditions of normal muscular activity by vasodilator phenomena which are often regarded as effects on capillaries, possibly due to the products of the muscular activity. If histamine and adrenaline also owe their vasodilator effects to action on capillary walls, it might be expected that this would often be obscured in the skin by their stimulant effect on arterial muscle. Especially would this be so in the case of adrenaline, reproducing in the arterioles of the skin the effects of their rich supply of sympathetic vasoconstrictor nerves. In the muscles, on the other hand, a vasodilator effect on capillaries might be expected to be more usually preponderant. The facts are again, therefore, in accordance with the supposition of a capillary-dilator effect. On this supposition we should expect the contrast between the response of skin and muscle to be greater in the case of adrenaline, with its intense arterio-constrictor action, than in that of histamine, with its weaker effect on arterial muscle. In several of our experiments this expectation also has been realised, though the difference is not obvious in Fig. 15.

Hunt mentions and illustrates an experiment in which the volume response of the fore limbs to acetyl-choline was recorded, one being intact and the other skinned. He found that the intact limb showed a much more pronounced expansion than the skinned muscles. Similarly he found that the venous outflow from a cutaneous vein underwent a much greater proportionate increase than that from a muscular vein when acetyl-choline was given. It will be obvious from what has been said above that this reversal of the relation between dilator effects in skin and muscle seen with adrenaline and histamine is in harmony with our view that the effect of acetyl-choline is due to arterial dilatation. We have only made one experiment on the point, the volume changes of a skinned limb being compared with those of the opposite whole limb, both having their nervous connexions intact. There was no difference between the response of the two limbs to acetyl-choline such as to indicate a greater effect on skin arteries than on muscle arteries; on the other hand there was no indication that the muscular vessels were preferentially affected, as is so frequently the case with adrenaline and histamine. Hunt's experiments, however, seem to demonstrate that conditions of relative

tonus do occur in which the skin vessels show the more pronounced dilatation with acetyl-choline.

Other features of the distribution of the vasodilator effects of these substances are of more doubtful significance. Dale observed erection of the cat's penis after a large hypodermic dose (20 mgm.) of acetyl-choline, and such an effect is certainly not produced by histamine under similar conditions. Hunt recorded the volume of a dog's penis with the plethysmograph and observed expansion with a small dose (0·02 mgm.) of acetyl-choline intravenously. Since the arteries are said to open directly into the cavernous spaces of the erectile tissue, without the intervention of true capillaries, this may be regarded as an unmixed arterio-dilator effect. There is, however, a yet unexcluded possibility that the effect may be central in origin.

V. Effects of local application of histamine.

We made several attempts to measure changes in the calibre of blood vessels—arterioles and capillaries—in the mesentery by direct observation with the microscope, histamine solution being directly applied to the area under observation. The results were not sufficiently convincing to ourselves to make it desirable to record them. Later experience of the ease with which this vasodilator action is impaired, by temporary interference with the circulation through an organ or exposure to cold, made it clear that satisfactory observations of this kind would need a specially elaborated technique. Results of some significance, however, can be obtained by simple observation with the naked eye of the effects of local application. If a solution of histamine (0·01–0·1 p.c.) in warm saline is brushed across a loop of the intestine of a cat under ether, the local effect on the circulation is complicated by the contraction of the muscular coats of the intestine which the histamine excites. This can be prevented by first painting the whole loop with 0·01 p.c. adrenaline, which inhibits the contractions of the intestinal muscle. The loop acquires a somewhat bluish pallor under the action of the adrenaline. If a drop of 0·1 p.c. histamine solution is then streaked across the loop with a soft brush, the bowel at the line of application becomes gradually red in comparison with that on either side. The reddening had the appearance of a general capillary flush, but the difficulty under the conditions of distinguishing the smallest arterioles from venules made it impossible to say whether the smallest visible arteries were involved in the dilatation. The smallest vessels clearly recognisable as arteries were certainly not dilated.

A clearer result was obtained with the cat's pancreas, which has few or no vessels visible to the naked eye on its surface. The surface of the organ has a very uniform pinkish tint, varying in depth in different animals according as the cells of the acini are loaded with secretory granules or discharged. A half loaded gland shows the effects well. If a pledget of cotton-wool soaked in 0·1 p.c. histamine solution is laid gently on the exposed pancreas, left for 10–20 seconds and then removed, the surface of the gland at the point of application shows a conspicuous diffuse red flush in comparison with the uniform pink of the rest of the pancreas. It seems to us unlikely that such an effect is due to histamine soaking into the gland and locally dilating arterioles. Further evidence of capillary involvement is obtained by allowing the pledget soaked with histamine solution to remain on the gland for 5 or 10 minutes. On now removing it, it is found that the gland underneath it, and in a zone extending a varying distance on either side of it, has become to a pronounced degree œdematous, so that the lobules appear clearly separated and as if embedded in a colourless transparent jelly. At a little distance from the point of application the gland is unaffected. Clearly there has been a local excess of transudation of lymph; the second stage of the effect of histamine has been the production of an abnormal permeability of the capillary walls. This seems to us to give support to the suggestion that the primary local hyperæmia is also due to an action on the capillaries. In the rabbit, in which histamine has no vasodilator action when given intravenously, we observed no effect from local application to the pancreas. The local effect of histamine on the cat's pancreas is clearly analogous to its effect on the lightly scarified human skin, as described by Eppinger(22) and more recently by Sollmann and Pilcher(23). The latter observers found that in solutions as weak as 1 in 100,000 histamine, applied to a scarified spot, caused local erythema terminating in the production of an urticarial wheal. They point out that other vasodilators, such as nitrites, have no such effect, and after a discussion of possibilities, conclude, we think rightly, that the urticaria is due to a specific effect on the permeability of the capillary walls.

VI. Perfusion of surviving organs.

The results of the experiments described in the foregoing pages, though they were often complicated and difficult of interpretation, had appeared to point consistently in one direction. Each of the anomalies in the vasodilator actions of histamine and adrenaline, each of the points

in which their actions differed from the more conventional vasodilator action of acetyl-choline, had seemed in turn to find an explanation, if we could suppose that the action of both presented a complex of inhibition of capillary tone and stimulation of arterial tone. Another anomaly of the vasodilator action of these substances remained to be considered—the fact that it had hitherto proved impossible to demonstrate it under conditions of artificial perfusion, although, as we have here shown, it is a purely peripheral effect, independent of the integrity of any nervous mechanism. It is obvious that this also finds its explanation in the above conception if we can suppose that the reactivity of the capillaries is less easily preserved under the conditions of artificial perfusion than that of arterial plain muscle. This is in itself a reasonable supposition, but we were anxious to put our conception to a more direct test. By varying the method of artificial perfusion we have found a combination of conditions which enables us with certainty to obtain the vasodilator effect of histamine in the vessels of the surviving organ, and, further, to make an anatomical separation between the vessels which, under those conditions, it dilates and those which, under identical conditions, it constricts.

The ordinary procedure for perfusing an isolated organ has more than one feature which, in the light of our observations on the conditions favouring the vasodilator effect of histamine *in vivo*, might be held responsible for previous failures to demonstrate it in perfusion experiments. In the first place we found that after a temporary interruption of the circulation the effect disappeared, and was only slowly recovered when the normal circulation was restored. In the ordinary perfusion experiment the vessels of the organ are ligatured, cannulæ are inserted, and the blood is washed out with saline before the artificial circulation is begun. In the second place the customary use of whipped blood is open to criticism for our purpose, since, as Brodie(24) showed, blood so treated has itself a complex activity, including effects which appear to belong to the same general type as that which was later ascribed to histamine, so that an organ perfused with whipped blood might be already in a condition of maximum response to this type of vasodilator effect, and capable only of exhibiting the associated constrictor response with a further dose of this class of poison. A third possibility, that the vessels might need the tonic effect of nervous impulses to enable them to show this type of response, had been excluded by our experiments on the effect of cutting the nerves to a limb in the living animal; but in the living animal, in the absence of nervous control, there is clearly some

influence which maintains a vascular tone, as shown by the tonic condition of the vessels in a limb denervated by degeneration. The presence of adrenaline in the circulating blood seems to provide the immediately obvious explanation; but the difficulty at once arises that small doses of adrenaline have a vasodilator action, which is especially pronounced in the vessels of a denervated organ. The conception of a tone maintained by adrenaline and relaxed by a small extra dose of the same substance is certainly difficult. We may reserve its fuller discussion till a later stage. There is one point, however, with which we must deal at once, since it is involved in the procedure we are about to describe. It will be seen that we use the addition of adrenaline to the perfusion fluid as a means of raising the vascular tone and giving scope for vasodilator action. The objection may possibly be raised, that in earlier sections we have given reason for believing, from the analogy of its action with that of histamine, that adrenaline produces its vasodilator effect by action on capillary walls; and it may be suggested that by adding it to the perfusion fluid we should, if this idea is accepted, be producing a state of capillary relaxation which, again on our view, should be unfavourable to the vasodilator action of histamine. It is necessary, therefore, again to emphasize the fact that this conception, so far as it applied to the effect of adrenaline, was concerned with the sudden effect of very small doses. There is good reason to believe that the constrictor effect of any but the smallest doses of adrenaline involves the capillaries as well as the arterioles. The maximum purely depressor dose of adrenaline for the average cat is about 0·005 mgm. Making the rather small allowance of 100 c.c. of blood for such a cat, and assuming that the whole dose remains in the blood, and reaches the site of its action intact, this gives a maximum rise of adrenaline content in the blood of 1 part in 20 millions as the result of such an injection. The concentrations used in our perfusions have been from 1 in 10 millions to 1 in 1 million, and this has been not momentarily attained, but steadily supplied, subject only to the slow diminution occurring during the passage of the whole perfusion mixture through the isolated organ. To obtain the effect which we required we have, therefore, found it necessary to use adrenaline in a maintained concentration much above the maximum at which, when momentarily attained, its vasodilator action appears *in vivo*. Cotton, Slade and Lewis(24) on the human arm with circulation arrested by compression of the artery, obtained effects by local injection of dilute adrenaline (1 in 30,000) which they could only explain by a direct constrictor action on capillary walls, and Langley(26) previously from ocular inspection

on local application suggested an action of adrenaline on certain capillaries.

Another point for investigation, suggested by our experiments *in vivo*, was the effect of oxygen supply. The possible bad effects of whipped blood might be avoided by using Ringer's solution as perfusion fluid, giving it the requisite viscosity and content of osmotically active colloid by addition of 6 p.c. gum arabic, as recommended by Bayliss. In doing so we should introduce another factor, that of imperfect oxygen-carrying power, owing to elimination of red corpuscles. This, however, might afford us a further opportunity of discriminating effects on arterial muscle from those on capillary endothelium, which, from the intimacy of its relation to the tissues needing a free supply of oxygen for their metabolism, would probably show earlier impairment of activity from deficiency of oxygen supply. Lastly, by suitable methods of arranging cannulæ, we could eliminate the usual interval between cessation of natural and commencement of artificial circulation. After the first few experiments we have always ensured this practical continuity of circulation, but it has made no perceptible difference in the results. The method of ensuring such continuity has been used in principle before (cf. Bainbridge and Evans(27), Richards and Plant(28)). The perfusions have been carried out on legs and on portions of small intestine. For perfusion of a leg the cannulæ have been inserted into the central ends of the external iliac artery and vein of the other side, the vessels being clamped between the cannulæ and the aorta and vena cava respectively. The aorta was tied beyond the origin of the external iliac artery, all branches of the external iliac artery and vein were tied, and also the profunda femoris vessels. Branches of the aorta and vena cava were tied up to a point above the origin of the inferior mesenteric artery, and the lumbar and abdominal muscles mass-ligatured, as described on p. 137. The cannulæ being connected with the perfusion apparatus, ligatures were drawn tight round the aorta and vena cava near the origin of the iliac vessels, and the clamps on the iliac artery and vein, into which the cannulæ had been inserted, were simultaneously removed. The artificial circulation through the femoral vessels of the opposite side thus began simultaneously with the cessation of the natural circulation. In one case, in which hirudinised blood was used, the cat furnishing the leg for perfusion was also hirudinised and the artificial flow was begun before the aorta and cava were tied, so that not even a momentary interruption of the blood-flow occurred.

Similar arrangements were made for the loop of intestine. The

arterial cannula was in this case inserted into the headward end of the aorta, just below the origin of the inferior mesenteric artery. All branches of the aorta above the cannula, up to and including the cœliac axis, were tied. All branches of the inferior mesenteric artery except those supplying the chosen loop of bowel, and all branches of the portal vein except those draining it, were systematically ligatured, and the stomach, the remainder of the small intestine, the spleen, the large intestine were removed after suitable ligaturing. The loop of bowel under observation, still with its normal circulation, was arranged in the plethysmograph, and the cannulæ connected to the perfusion scheme. When all was ready the aorta was ligatured just above the inferior mesenteric artery, and the clamp above the perfusion cannula simultaneously removed. The portal vein was then rapidly incised and an outflow cannula tied into it. When hirudinised blood was used the cat was also hirudinised and perfusion begun before the natural circulation was interrupted, as in the similar experiment on the leg. The effect of interruption of the circulation being thus eliminated, the considerations discussed above had suggested three possibilities for the conditions necessary for demonstrating the vasodilator action of histamine on the artificially prepared organ.

1. It might be possible to demonstrate it if an adequate tone of the vessels were produced by adding adrenaline to the perfusion fluid.

2. The necessary condition might be found in an adequate oxygen-carrying power of the perfusion fluid. We could confer this, and eliminate the defects of whipped blood discussed above, by adding washed red corpuscles to the Ringer's solution or using pure hirudinised blood for perfusion.

3. Both the oxygen-carrying power conferred by corpuscles, and the production of adequate tone by suitable addition of adrenaline, might be necessary.

All three possibilities have been tested on both hind limb and bowel segment, and under each set of conditions we have tested the action of histamine and of acetyl-choline. The conditions were obviously inapplicable to the vasodilator action of adrenaline. Yet a fourth possibility has been tested by using a leg in which the blood vessels had acquired an abnormal reactivity and a natural tendency to tone as a result of degeneration of the nerves to the leg, after section sixteen days previously. The effects of acetyl-choline and histamine were tested during perfusion of this leg with hirudinised blood and with gum-Ringer solution, with and without addition of adrenaline in each case.

In these experiments on perfused organs the dose of the substance

being tested was injected by a fine hypodermic needle, pushed through the rubber tubing of the perfusion scheme a short distance above the cannula in the artery. To avoid possible small errors due to changes of viscosity in the fluid reaching the artery, or momentary reduction of adrenaline concentration when this substance was present in the perfusion fluid, doses of histamine were always made up in a sample of the fluid actually being used for perfusion. This could not be done in the case of acetyl-choline when blood was being used, owing to the rapid hydrolysis and consequent loss of activity which it undergoes on standing in contact with blood. The volume of the injection, however, was very small (usually 0·1 c.c.), and the striking nature of the effects of this substance made it impossible in any case to attribute them to the trifling changes of viscosity which might momentarily occur as the result of such an injection.

The following were the results obtained. The effect of acetyl-choline was only tried in the cases mentioned.

1. *Perfusion with oxygenated Ringer's solution containing* 6 *p.c. gum arabic.*

(*a*) Normal leg—Histamine—(in doses from 0·002 mgm. up to 0·1 mgm.) caused contraction of the limb in the plethysmograph and retardation of outflow from the vein.

(*b*) Loop of bowel—Histamine caused the same effect as in the limb—contraction of volume and retardation of venous outflow.

2. *Perfusion with oxygenated Ringer's solution containing* 6 *p.c. gum* + 1 *in* 10 *million to* 1 *in* 1 *million adrenaline.*

(*a*) Normal leg—Histamine—contraction and retardation of outflow. The doses used were 0·01 to 0·03 mgm. Smaller doses had no effect. In the one experiment where very dilute adrenaline (1 in 10 millions) was used, which under these conditions made no difference in the rate of perfusion, 0·02 mgm. of histamine produced a greater reduction of leg volume and retardation of outflow than it had caused earlier in the experiment, where plain gum-Ringer with no adrenaline had been perfused.

Fig. 16 illustrates another experiment. In this case 1 part of adrenaline in $2\frac{1}{2}$ millions was added and the perfusion pressure had to be raised to about 155 mm. of mercury to secure an adequate perfusion rate, showing that a high tone of the vessels had been produced. Nevertheless 0·03 mgm. of histamine still causes contraction of the leg and retardation of the outflow. A slight rise of the perfusion pressure, indicating increased resistance to the flow through the leg, is perceptible as the result of injecting histamine. (It should be remembered in considering this and the following figures that the perfusion is carried out under a static head of pressure with rhythmic interruptions. The changes of pressure recorded are, therefore, small in proportion to the changes of resistance in the organ.)

In only one instance was there any deviation from this type of result. In this experiment, in a preliminary perfusion of the limb with gum-Ringer without adrenaline, 0·002 mgm. of histamine had caused a pronounced constriction of the limb, with a relatively very small change in the rate of outflow, while 0·01 mgm. caused contraction of volume and slower outflow as usual. Adrenaline was then added in the proportion of 1 to 5 millions, and the perfusion pressure raised to keep the flow practically constant. 0·01 mgm. of histamine now had a puzzling effect. The volume of the limb diminished as before; the

outflow, however, showed no retardation, but a late and quite obvious acceleration, which showed no tendency to pass off. After an interval of some minutes the dose was repeated with a similar effect—contraction of the limb, and later a further persistent acceleration of outflow. Still later 0·1 mgm. of histamine caused the usual contraction of volume and slowing of outflow. This result is quite anomalous and cannot be explained by any type of vasodilator action. The limb showed a great tendency to become œdematous and increased continuously in volume throughout the perfusion. We found that the plethysmograph fitted rather tightly, and it is possible that the small shrinkage of the limb caused by histamine released fluid which had been accumulating in skin veins, and thus obscured the direct effect of histamine on outflow.

(*b*) Loop of bowel. The addition of adrenaline up to 1 part in 1 million, necessitating a greatly increased perfusion pressure to maintain an adequate flow, reduced the constrict-

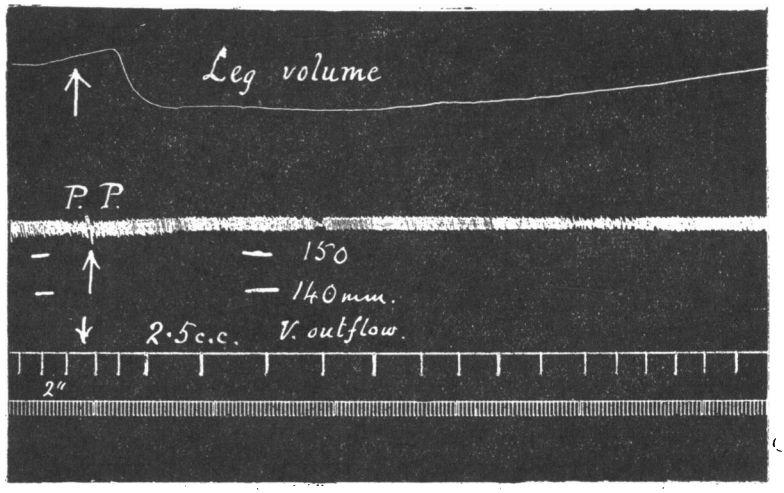

Fig. 16. Perfusion of leg with gum-Ringer and adrenaline (1 in 2½ millions). Effect of 0·03 mgm. histamine.

ing and retarding effect of smaller doses of histamine (0·002 mgm.), but the smallest effective doses remained constrictor in effect, as with the leg.

(*c*) Denervated leg. Histamine caused a similar constriction and retardation of outflow to that which it caused in the normal leg.

Acetyl-choline (0·01 mgm.) caused expansion of the leg and accelerated outflow.

3. *Perfusion with hirudinised blood.*

(*a*) Normal leg—Histamine (0·02 mgm.) caused constriction and retardation, with rise of perfusion pressure.

(*b*) Loop of bowel—Histamine (0·002 mgm.) caused contraction of volume, rise of perfusion pressure and retardation of outflow (Fig. 17 A).

(*c*) Denervated leg—Histamine (0·01 mgm.) caused contraction of volume, rise of perfusion pressure (slight) and retardation of outflow (Fig. 18 A). Acetyl-choline (0·001 mgm.) caused expansion of volume, fall of perfusion pressure (well marked) and accelerated outflow (Fig. 18 B).

4. *Perfusion of hirudinised blood with adrenaline added.*

(*a*) Normal leg. Adrenaline 1 in 10 millions. Perfusion pressure 180 mm. of mercury. Histamine (0·1 mgm.) caused expansion of the limb, fall of perfusion pressure, and accelerated outflow (Fig. 19).

(*b*) Loop of bowel. Adrenaline 1 in 1 million. Perfusion pressure had to be raised from

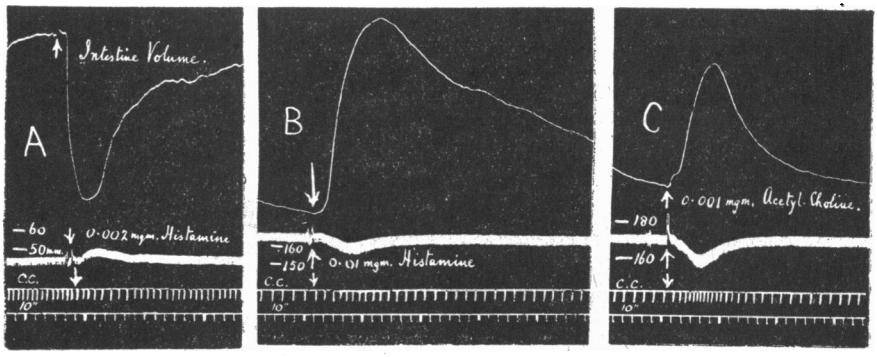

Fig. 17. Perfusion of intestinal loop with hirudinised blood. Oncometer, perfusion pressure, and venous outflow. *A* before, *B* and *C* after addition of 1 part of adrenaline to 1 million of perfusing blood.

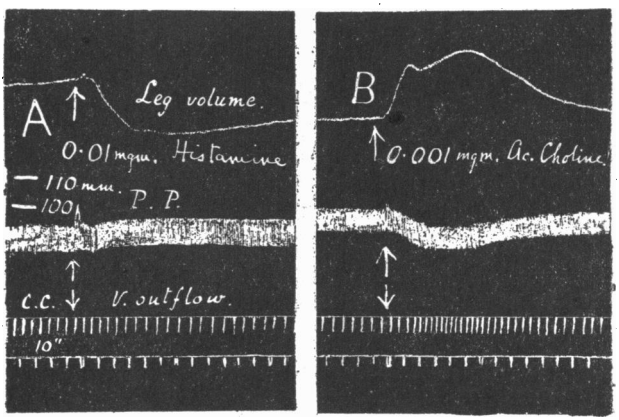

Fig. 18. Completely denervated leg perfused with hirudinised blood (no adrenaline added).

about 45 mm. (Fig. 17 A) to about 165 mm. (Fig. 17 B) to secure adequate perfusion rate after addition of adrenaline.

Histamine (0·01 mgm.) caused large expansion of volume, fall of perfusion pressure and acceleration of outflow (Fig. 17 B).

Acetyl-choline (0·001 mgm.) caused expansion of volume, fall of perfusion pressure and acceleration of outflow (Fig. 17 C).

Note that acetyl-choline, though in this dose it causes a smaller volume-increase than 0·01 mgm. of histamine, causes a more pronounced fall of perfusion pressure and a greater acceleration of outflow.

(c) Denervated leg. By a mistake the proportion of adrenaline added (1 in million) was much too great, so that even with the highest pressure obtainable with our hydrostatic perfusion the flow through the organ was very slow. Nevertheless the effects with histamine and acetyl-choline were unmistakeable; both now caused expansion of the limb, fall of perfusion pressure, and accelerated outflow.

5. *Perfusion with Ringer's solution containing 5 p.c. gum + washed red corpuscles of cat's blood + adrenaline 1 in 5 millions.*

Normal limb. Histamine (0·02 mgm.) caused expansion of the limb, fall of perfusion pressure and acceleration of outflow.

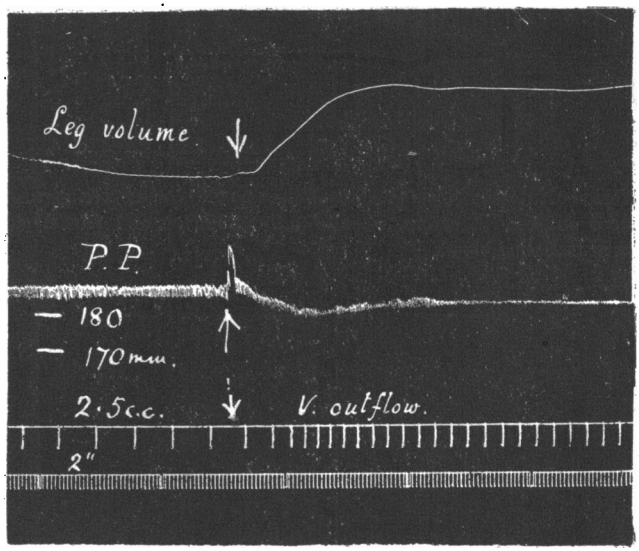

Fig. 19. Perfusion of normal leg with hirudinised blood to which 1 part of adrenaline in 10 millions has been added. Effect of 0·1 mgm. histamine.

The results of these experiments, which have been presented in this summarized form, show a striking difference between the behaviour of histamine and acetyl-choline. It will be seen that acetyl-choline regularly caused changes indicative of vasodilatation, in all conditions under which it was tested. All that was necessary for the exhibition of this action was the pre-existence of adequate tone in the vessels to give scope for its display. Thus when gum-Ringer was perfused, with the addition of a small quantity of adrenaline to produce tone of the vessels, acetyl-choline showed a well-marked vasodilator action. When the tone was adequate in the absence of adrenaline, as in the denervated limb per-

fused with blood, acetyl-choline produced again a pronounced vasodilatation.

With histamine the case is far otherwise. It will be seen that we tried all combinations with this substance, using oxygenated gum-Ringer and blood, each with and without addition of adrenaline, for perfusion of the hind leg, with its vessels in normal condition or rendered abnormally reactive by degenerative denervation, or a loop of bowel. In no experiment with Ringer's solution and gum did we obtain any effect on the volume of the organ but contraction as the result of injecting histamine, even when adrenaline was added to the perfusion mixture in such a proportion that a high pressure was needed to maintain an adequate rate of perfusion. In only one case, in which there was evidence of technical error, were the effects on the perfusion pressure and outflow other than those of simple vasoconstriction—rise of pressure and retarded outflow. Similarly, in experiments in which plain blood was perfused, we never obtained with histamine any effects except those of simple vasoconstriction—contraction of volume, rise of perfusion pressure and retarded venous outflow. This was the result even with the denervated limb, in which perfusion with hirudinised blood was begun before the natural circulation was stopped. The animal had been hirudinised, and the volume-reaction of the limb to histamine was tested just before the perfusion was started, and was found to be simple dilatation. As soon as possible after the change from natural to artificial circulation was completed, we tested again the reaction to histamine and observed simple constriction (Fig. 18 A). On the other hand, on organs perfused with blood to which adrenaline had been added histamine never produced any other effects than those of uncomplicated vasodilatation—expansion of volume, fall of perfusion pressure, and accelerated venous outflow.

The experiment on a loop of small intestine, illustrated by Fig. 17, shows especially well the change in response to histamine caused by the addition of adrenaline to perfused blood. In the living animal it is the larger doses of histamine which are specially liable to produce vasoconstrictor effects, even when smaller doses cause only vasodilatation. In this case a pure constrictor effect is produced by 0·002 mgm. of histamine when plain blood is being perfused; after adrenaline has been added a dose five times as large (0·01 mgm.) causes pure vasodilatation.

It is clear, then, that the vascular tone which acetyl-choline relaxes is easily evoked, and is maintained under a variety of conditions. Before these experiments were undertaken the dilator effect of acetyl-choline

had been demonstrated by Dale, and more recently by Hunt, on the rabbit's ear simply perfused with cold Ringer's solution. The dilator effect of histamine on the cat's blood vessels is, on the contrary, a singularly evanescent and elusive phenomenon, needing a special combination of conditions for its demonstration in the perfused organ. The question arises, what is the constituent of blood which must be present in the perfusion fluid in order that adrenaline may be able to produce the vascular tone relaxed by histamine? This is answered by the experiment quoted above under heading (**5.**), in which instead of blood we used a gum-saline mixture to which we added thoroughly washed red corpuscles. This mixture was quite as effective as whole blood in enabling adrenaline to produce a tone which histamine would relax; so that the constituent of blood needed for the effect is the red corpuscles. This we take to mean that the oxygen carrying capacity of a saline solution is inadequate for the purpose.

We find it impossible to conceive of two different kinds of tonus in the same contractile structure, the one, which is relaxed by acetyl-choline but intensified by histamine, being developed under the conditions provided by perfusion with oxygenated saline, and the other, which histamine also relaxes, being produced by adrenaline only when abundant oxygen is provided by the presence of red corpuscles. The only interpretation of the facts which seems to us at all adequate is the supposition that the two substances are acting on different contractile elements in the system of blood vessels. One of these, which is relaxed by acetyl-choline, but stimulated to contraction by histamine, in its resistance to the adverse conditions of ordinary perfusion, and in its ready acquisition of tone with adrenaline perfused in saline solutions, has the properties of arterial plain muscle. The other, which histamine relaxes, is so dependent on an adequate oxygen supply that it does not acquire tone with adrenaline unless red corpuscles are present. Again the walls of the capillaries, with their intimate physiological relation to the oxygen-using tissues, and their susceptibility to the products of imperfect metabolism, seemed to us to be clearly indicated.

When records of the vasodilator actions of acetyl-choline and histamine on the same organ are compared in detail, there is a suggestive difference, well shown in Fig. 17. Attention has already been called to the larger effect of histamine on organ-volume in this experiment, associated with a smaller effect on perfusion pressure and venous outflow than those produced by the dose of acetyl-choline which follows. In this experiment a dose of amyl nitrite was subsequently given, and

the proportion between the volume effect and those on pressure and outflow resembled that seen with acetyl-choline, and was similarly contrasted with that obtaining in the effects of histamine. This kind of disparity in the effects of histamine and acetyl-choline has been regularly present in experiments in which the vasodilator effects of both were produced. Histamine, therefore, appears to act on some part of the system of blood vessels relaxation of which has a relatively large effect on the volume of the organ, and a relatively small effect on the resistance to the blood flow. This again is in harmony with the view that it acts on the capillaries with their much larger total sectional area and capacity; the arterioles, with their more important role in determining the resistance to blood flow, being concerned in the vasodilator effects of acetyl-choline, as in those of nitrites.

Since so many different indications appeared to point in the one direction, it was highly desirable to devise an experiment in which the action of the two substances could be tested on the different types of blood vessel separately. We have already described some experiments made by the method of direct application, with results which, though they lend support to the view under consideration, were not decisive. The most conclusive test would be furnished by a perfusion of capillaries only, without intervention of arteries. This has not proved practicable, but we have found no difficulty in carrying out the complementary experiment of perfusion through arteries, including almost the smallest branches still recognisable as such, with no capillaries.

The arteries chosen were the superior mesenteric artery with its branches and their fine ramifications as they pass on to the small intestine. By cutting through the mesentery close to the wall of the intestine, and allowing the blood to drip from the ends of the small arteries thus severed, a preparation is obtained in which perfusion can be carried out through arteries only, and in which the outflow measured is from comparatively fine arterial branches. The arterial cannula is inserted into the aorta as for a perfusion of an intestinal loop. The mesentery is divided above and below the area of arterial distribution to be used for the perfusion. When all is ready the aorta is tied above the origin of the superior mesenteric artery, this artery and its branches are then rapidly washed through with oxygenated Ringer's solution, the mesentery is cut through with sharp scissors as close as possible to the wall of the bowel, and perfusion with hirudinised blood is immediately started. (If the preliminary wash-through with Ringer's solution was not carried out, we found that even hirudinised blood did not prevent the

outflow from the small cut arteries becoming obstructed by minute clots.) The preparation is then cut free and suspended from the arterial cannula in a cylindrical glass chamber. The upper end of this is closed by a cork through which pass the cannula and a thermometer, and at its lower end it is narrowed funnel-wise into a tube from which the blood, dripping from the cut edge of the hanging bunch of mesentery, issues in a regular series of drops or a small unbroken stream. The chamber is kept at uniform temperature by an electric glow-lamp placed at an adjustable distance from it, and the preparation is thus protected from both evaporation and cooling. The outflow is recorded in the same way as the venous outflow in our perfusions of whole organs. Doses, as before, are injected into the rubber tubing just above the arterial cannula.

The results are shown in Fig. 20. (*a*) and (*b*) show the effects of small doses of histamine and acetyl-choline when plain hirudinised blood was being perfused. The arteries had little tone and with a low perfusion pressure (65 mm.) the outflow was rapid. Histamine (0·02 mgm.) produces a definite retardation, acetyl-choline a slight acceleration of the outflow. The actual rates, as measured, are 13·4 c.c. per minute before, 16·5 c.c. per minute after injection of 0·001 c.c. acetyl-choline. Adrenaline in the proportion of 1 to 1 million was then added to the perfused blood. The perfusion pressure was raised to about 158 mm. to maintain the rate of perfusion. Histamine (0·01 mgm.) now produces a comparatively weak but still perceptible retardation of flow, 8·5 c.c. per minute being reduced to 7·75 c.c. per minute (Fig. 20 (*c*)). A smaller dose (0·002 mgm.) produced no definite effect, while a larger dose (0·1 mgm.) caused a decided constriction, as shown both by rise in perfusion pressure and retardation of outflow, 10·1 c.c. per minute being reduced to 6·8 c.c. per minute (Fig. 20 (*e*)).

Acetyl-choline under these conditions causes a very obvious dilatation of the arteries, as shown both by increased outflow and fall of the perfusion pressure (Fig. 20 (*d*) and (*f*)). Amyl nitrite (0·01 c.c. emulsified in gum-saline) produced a similar effect to that produced by 0·002 mgm. of acetyl-choline.

Under conditions, therefore, which enable it to produce vasodilatation in the loop of small intestine, histamine causes simple constriction of the arteries supplying that loop, down to their finest anatomically separable branches, while acetyl-choline still produces vasodilatation of arteries thus separated.

The dilator effect of histamine must be on vessels which lie beyond the small arteries, and we are thus led by direct evidence to locate this

action on the capillaries, as previously by indirect evidence from the conditions necessary for its demonstration. Of these conditions the need of red corpuscles for the maintenance of capillary tone is intelligible on the lines already indicated. The need for adrenaline requires some further consideration. We must make it clear, in the first place, that we

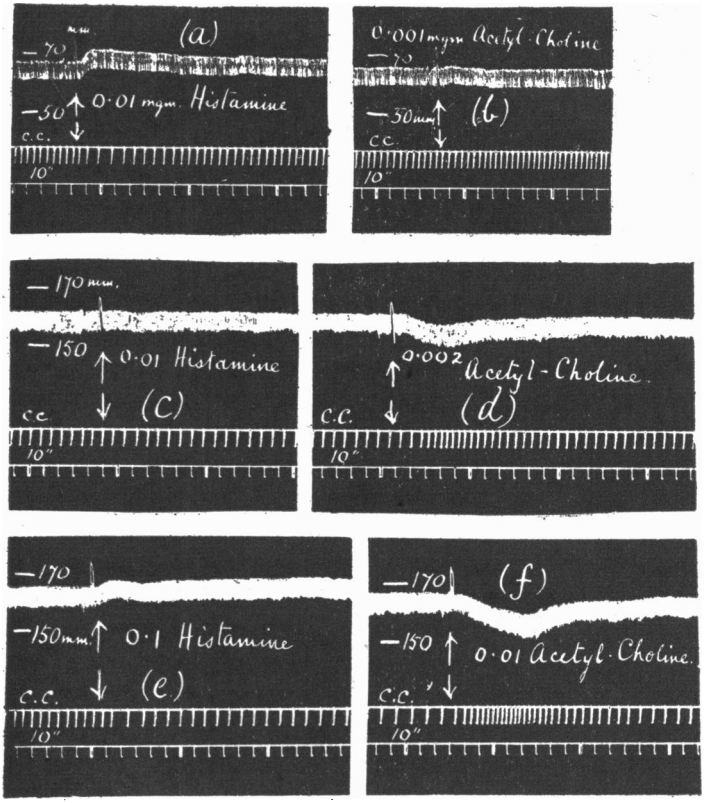

Fig. 20. Perfusion of branches of superior mesenteric artery with hirudinised blood. (*a*) and (*b*) *before*, (*c*), (*d*), (*e*) and (*f*) *after* adding 1 part of adrenaline to 1 million of blood. Perfusion pressure and outflow from cut arterioles.

have no evidence that adrenaline is the only substance capable of producing in the perfused organ the vascular tone which histamine inhibits. It is the only substance which we have hitherto tried for this purpose. The point of interest is rather that any such substance should be needed. We have described above an experiment in which we ensured the presence of all the known conditions necessary for an intense vasodilator action by

histamine in the living animal. We used a limb in which the nerves had been cut sixteen days previously, and assured ourselves that, while its natural circulation persisted, it dilated readily with histamine. Lest hirudin in the perfused blood might introduce an unfavourable factor, we injected hirudin into the animal and found that the dilator reaction of the leg persisted. Lastly, lest even a momentary stoppage of circulation should be unfavourable, we began perfusion, from the iliac vessels of the opposite side, before we interrupted the normal circulation. Nevertheless, by the time we were able to make the test, the vasodilator action of histamine in the perfused limb had already vanished, and only a constrictor action remained. Arterial tone was still good, as shown by the vasodilator action of acetyl-choline. The dilator action of histamine did not reappear, the capillary tone was not restored, if our conception is correct, until adrenaline was added to the blood. Why did the change, without even momentary break, from the natural circulation of hirudinised blood to the artificial perfusion of hirudinised blood lead to the loss of capillary tone? Since we are dealing with an organ freed by degeneration from all nervous connexion, the question can be put in another form. What is the substance present in the naturally circulating blood, but absent from blood a short time after it has been shed, which maintains capillary tone in the absence of nervous control? Adrenaline is the immediately obvious suggestion; our observation, that addition of adrenaline to the perfused blood would restore the vasodilator effect of histamine, is at least in accordance with such a suggestion, if it gives it no positive support. When we come, however, to consider how adrenaline, in the concentration in which it exists in the peripheral blood, can play such a part in the living animal, we are met by a difficulty. In the cat, which is the animal on which all our experiments have been made, adrenaline itself, when injected in doses up to about 0·005 mgm., produces a vasodilator action, and we have given reason for believing that this action is of the same type as that of histamine, and therefore presumably an action on capillaries. But experiments in which the output of adrenaline from the suprarenal glands was measured in anæsthetised cats showed that the amount entering the vena cava varied from 0·0008 to 0·0028 mgm. per minute in cats of 2 to 3 kilos body weight(29). Can it be supposed that this slow, steady addition of adrenaline to the circulating blood produces a state of tone, which is temporarily relaxed by suddenly throwing into the blood-stream a further dose of the order of that supplied by the natural secretion in the course of one or two minutes? The conception appears difficult, and is not rendered less so by the fact

that large doses of adrenaline (0·05 mgm. and upwards), suddenly injected, cause a simple increase of vascular tone—the vasoconstrictor, pressor effect usually recognised as the characteristic action of adrenaline. There is good reason for believing that capillaries as well as arterioles are involved in the vasoconstrictor action (cf. Langley(26), Cotton, Slade and Lewis(25)). This conception, however, of a tone produced by adrenaline, which an additional dose of the same substance may either slacken or intensify, according to the amount given, is by no means new to anyone who is familiar with the different types of effect produced by adrenaline after ergotoxine has been administered. When, after a dose of ergotoxine insufficient to paralyse completely the vasoconstrictor action of adrenaline, the blood-pressure has been allowed to return to a low level, the first effect of a moderate dose of adrenaline (say 0·05 mgm.) may be to cause a slight further fall, but this is succeeded by a long slow rise of pressure. A second similar dose, given during this rise, produces a more pronounced fall succeeded by a further rise of pressure; and in this way, by successive doses of adrenaline, each producing a more pronounced initial depressor effect, the blood-pressure may be raised to a high level, from which a conspicuous fall follows each injection of adrenaline of the order of 0·05 mgm., while a large injection (say 0·5 mgm.) still causes an immediate further rise. So that it is possible, under the abnormal conditions produced by sufficient ergotoxine to weaken without abolishing the constrictor action, to build up a vascular tone by adrenaline, which further doses may either relax or augment. There is, therefore, no insuperable difficulty in the supposition that, on a lower scale of dosage, the double action of adrenaline may be manifested, in the normal animal, by an analogous paradoxical effect, a further dose relaxing the tone which the same substance in steady low concentration has produced. The possibility needs further experimental exploration before it can be accepted as a satisfactory explanation of the phenomenon with which we are dealing; but it cannot be set-aside as inherently incredible, or contrary to all experience.

VII. Remarks.

This question of the maintenance of vascular and especially of capillary tone, apart from nervous control, is one possessing great possibilities of practical interest. The probability that some products of cellular activity produce a relaxation of capillary tone, such as we have here identified as the action of histamine, has long been recognised, and has frequently been associated with the increased rate of blood-flow

through an organ, accompanying acceleration of its metabolism (30). The appearance of substances, having what we call the "histamine" type of action on the vascular system, in blood subjected to mechanical violence or during the process of clotting (Brodie), and the fact that extracts of so many organs and tissues contain substances exhibiting this type of activity, must have some physiological significance. Barcroft and Piper (31) attributed the vasodilatation, occurring in the submaxillary gland after injection of adrenaline, to some product of the increased metabolism of the gland cells. It is perhaps significant that they found this action to be abolished when the gland was subjected to prolonged perfusion with cold saline, adrenaline then producing only its vasoconstrictor effect after the normal circulation had been restored. We have found that similar treatment of a leg abolishes the dilator action of histamine, and have attributed the change to depression of the reactivity of the capillaries. If it be the case that metabolic products with histamine-like action are constantly arising in the tissues, their production accelerated by every excitatory or injurious influence which intensifies metabolic activity, the capillary endothelium must as constantly be exposed to influences tending to relaxation of its tone. In a forthcoming paper, dealing with the action of large doses of histamine, we shall show how the simultaneous production of such action in an intense form throughout the body rapidly produces a condition of irremediable circulatory collapse, due to the stagnation of practically all the blood in the peripheral vessels. The continuous formation of metabolic products with histamine-like action must surely lead to a similar shock-like stagnation of blood in the relaxed capillaries, unless its influence were constantly neutralised and capillary tone upheld by nervous impulses, or by the continuous addition to the blood of some antagonistic principle. It seems to us that a more general investigation of the means by which this balance is normally maintained, and of the conditions leading to its upset in the direction of general capillary relaxation, is likely to give information having an important bearing on traumatic shock and allied conditions (cf. Cannon (32)). There is very little evidence concerning nervous control of capillary tone in mammalia, though there is some evidence of its existence in the frog. The possibility that maintenance and restoration of capillary tone may be among the most important of the functions of adrenaline is an attractive subject for speculation, but as yet no more.

A word may be added as to the relations between the vasodilator effects here under consideration and the different types of innervation of blood vessels. As pointed out by Dale, and more recently by Hunt,

the effects of acetyl-choline, like those of muscarine or pilocarpine, have in most directions an obvious likeness to those of the nerves called parasympathetic in Langley's classification of the autonomic system. But its general dilator action on systemic arteries has no parallel in parasympathetic innervation. Hunt tests the possibility of an association between vasodilatation by acetyl-choline and the antidromic action of sensory nerves. He rejects it on the ground that atropine does not abolish the latter effect, while it immediately suppresses that of acetyl-choline. The argument has not much weight, as he admits, since the vasodilator effect of the chorda tympani, which acetyl-choline obviously simulates, is but little affected by moderate doses of atropine. We have frankly abandoned the attempt to establish a connexion between the vasodilator effects of the three substances which we have specially studied and any system of innervation. From analogy with its other effects we should expect the vasodilator action of adrenaline to be represented in the sympathetic innervation of the vessels. The evidence for the existence of vasodilator effects of true sympathetic nerves is, however, scattered and meagre; the dilatation of the coronary arteries, the flushing of the mucous membrane of the dog's mouth, described by Dastre and Morat(33) as caused by stimulating the cervical sympathetic nerve, and certain relatively weak vasodilator effects of sympathetic nerves seen after ergotoxine, seem to be the only definite examples. On the other hand the vasodilator action of small doses of adrenaline so closely resembles in its distribution, and in the conditions favouring its demonstration, the more powerful effect of histamine, that it seems possible that the two substances have, in this direction, a common type of action, and that this is something additional and extraneous to the sympathomimetic action of adrenaline.

The only hint given by our experiments of connexion between innervation and the effects of these substances, is the enhancement of the effects of all three produced by denervation. We take this, however, simply to mean that the reactivity of the normally innervated vessels is restricted by the effect of tonic impulses from the nerve centres; only when these are cut off, and when the stimuli aroused by nerve section and by the earlier processes of degeneration have passed off, can the contractile elements in the blood vessels exhibit their unhampered response to these drugs. This view is supported by the fact that denervation similarly enhances the vasodilator effects of amyl nitrite and nitro-glycerine, which have never been associated with any type of innervation.

For the sake of brevity and emphasis we have throughout this paper

spoken of the vasodilator effect of histamine (and by analogy that of adrenaline) as due, in our opinion, to action on capillary walls; of the constrictor effect of histamine and the dilator effect of acetyl-choline as due to action on arterial muscle. It is important, therefore, to make it clear that no sharp distinction is warranted by our evidence. What we have actually on evidence is that the anatomically separable arterial branches are stimulated to constriction by histamine and adrenaline, to dilatation by acetyl-choline; that, therefore, the dilator effect of histamine which, under identical conditions, predominates in the whole organ, must be an effect on vessels more peripheral than these. The conditions necessary to produce this vasodilator effect support the view that it is mainly an effect on capillaries. The possibility is not directly excluded that it may spread on to the smaller arteries, overlapping with the vaso-constrictor action in such a way that there is a zone in which one or other action is the effective resultant according to dosage. At the same time there is no evidence of such overlap in the case of histamine, and such a conception would renew our original difficulty of reconciling a relaxing effect on arterial muscle with the stimulant action of histamine on all kinds of plain muscle, in any dose in which it is effective at all.

The simpler conception which regards the dilator effect of histamine as an action on capillary walls, and its action on the plain muscle of the arteries as being invariably to increase its tonus, accords better with the known facts. In the case of adrenaline the position is different. The evidence for the more peripheral location of its dilator action is indirect as yet, and there is evidence of overlapping of the two types of action, since the tone of the capillaries also is increased by larger doses and apparently by the smallest effective concentrations if maintained. It may well be that the vasodilator action of a sudden small injection of this substance affects both the capillaries and the smallest arteries, while the constrictor effect of a bigger dose spreads from the arteries to involve the capillaries as well. The reversal of its action on the lung vessels with increase of dosage (Tribe(34)) may possibly find its explanation along these lines. The whole question of the relation of these opponent effects of adrenaline needs further investigation; even the relation of the normal dilator action of small doses, to that produced by doses of any dimensions after an adequate amount of ergotoxine has been administered, requires clearer definition. In the case of acetyl-choline our evidence only entitles us to say that it possesses an inhibitor effect on arterial muscle which sufficiently accounts for its vasodilator action along conventional lines; it *may* directly relax capillaries as well, but we have no evidence of its doing so.

We are aware that the view which we have here put forward as to the manner of action of small doses of histamine necessitates some modification of the accepted teaching, which localises the peripheral resistance, by which the arterial pressure is maintained, almost wholly in the smaller arterial branches, and regards the resistance met in the capillaries as almost negligible from this point of view. Local variations of capillary tone have been described (cf. Roy and Graham Brown(19) who cite earlier literature) and have been credited with importance in directing the distribution of the blood under conditions of low blood-pressure (Langley(35)). So far as we are aware, however, the conception of the intrinsic tone of the capillary wall as playing an important part in regulating the general peripheral resistance, and maintaining the arterial blood-pressure, does not enter into any accepted system of physiological teaching. Yet this is implicit in the view which we have put forward; for the fall of arterial blood-pressure caused by a small dose of histamine has all the characteristics of a fall caused by the recognised agents for lowering the peripheral resistance. It is not a "volume" effect, due simply to an increased capacity of the system; if it were so the output of the heart must be lessened during the fall of blood-pressure, owing to diminished venous inflow, whereas it is somewhat increased. The question which arises is whether the accepted teaching, locating the peripheral resistance in the arterioles, and attributing variations in its strength entirely to variations in the tone of arterial muscle, is based on evidence so conclusive as to nullify the evidence which we have put forward. It does not seem to us that it is so. There is evidence that the total sectional area of the capillaries is very much larger than that of the larger arteries, though it may be doubted whether the measurement of the rate of flow through particular capillaries, exposed for microscopic observation, and the assumption that the general rate throughout the body is of the same order, can lead to a quantitatively correct estimate of the relation. There is evidence, again, that the pressure in capillaries accessible for its measurement is usually nearer that of the veins than that of the arteries. We find no evidence, however, to warrant the assumption that the steep fall of pressure in the arterioles suffers an abrupt flattening, becomes in fact almost horizontal, at a sharp line of demarcation between the smallest arterioles and the first capillaries. We find nothing to exclude the view that a general tone of the capillaries, if it exists, will play an important part in determining the peripheral resistance, and that a sudden relaxation of this tone will cause a fall of blood-pressure of the vasodilator type. It is, however, legitimate to suppose that such a relaxation would have more effect on the volume of

the organs involved, in proportion to the diminution of resistance effected, than a relaxation of the tone of the corresponding arterioles. Under the uncomplicated conditions of artificial perfusion we have shown that this expectation is realised. Whether this interpretation of the facts here recorded be correct or not, it is clear that the results obtained by artificial perfusion of isolated organs lead to an incomplete and misleading conception of the action of histamine and similarly acting substances, if the experiment is made in the ordinary way. Other factors are concerned in the action of these substances on the circulation than those which have hitherto been appreciated, and we are convinced that the physiological importance of these factors will be increasingly apparent.

SUMMARY.

Our main conclusions may be summarized as follows:

1. The vasodilator actions of histamine, adrenaline and acetyl-choline are purely peripheral effects on the blood vessels, independent of the integrity of any nervous connexion. The blood vessels are usually rendered abnormally sensitive to these actions by complete degeneration of the nerve-supply.

2. The vasodilator action of acetyl-choline is dependent on the state of tone and reactivity of the arteries.

3. The vasodilator actions of histamine and adrenaline undergo parallel variations with changes in the condition of the blood vessels. These variations are independent of the tone of the arteries, and both actions are favoured by a condition which we regard as indicative of tone and reactivity of the capillaries. The vasodilator actions of the two substances resemble one another further in their limitation to certain animal types and their relative prominence in the vessels of different organs.

4. The vasodilator action of histamine can be regularly demonstrated on artificially prefused organs of the cat if the perfusion fluid contains red blood-corpuscles and a small proportion of adrenaline. If either is omitted histamine produces only vasoconstriction, as in earlier experiments. Even when both are present histamine produces constriction of arteries separated from their peripheral distribution. Acetyl-choline produces dilatation in the perfused organ or separated arteries under the simplest conditions.

5. We conclude that the vasodilator effect of histamine, and probably that of adrenaline, are due to relaxation of the tone of the capillaries, while that of acetyl-choline is due to action on arteries. Histamine has

a constrictor effect on arteries; the better known vasoconstrictor effect of adrenaline probably involves both arteries and capillaries.

6. No exact line can be drawn at present as to the point where the characteristic arterial reaction gives way to the characteristic capillary reaction.

7. It is suggested that the current conception of the peripheral resistance to blood flow, as determined almost exclusively by the tone of the arterioles, allows too little importance to capillary tone as a factor.

8. No connexion has been established between the vasodilator actions of histamine and adrenaline and any system of nerves.

9. The possibility is considered that substances with histamine-like action are produced by activity or injury of the tissues, and the bearing of this on physiological vasodilatation and shock is discussed.

BIBLIOGRAPHY.

(1) Dale and Laidlaw. This Journal, **41**, p. 318, 1910; **43**, p. 182. 1911.
(2) Guggenheim. Therap. Monatsch. **26**, p. 795. 1912.
(3) Mautner and Pick. Münch. med. Woch. **62**, p. 1141. 1915.
(4) E. Mellanby. Quart. Journ. Med. **9**, p. 165. 1916.
(5) M. R. C. Memorandum. B. M. J. 1917, i, p. 381; Lancet, 1917, i, p. 502.
(6) Hartman and Fraser. Amer. Journ. Physiol. **44**, p. 353. 1917.
(7) Dale. Journ. Pharm. and exp. Ther. **6**, p. 147. 1914.
(8) Hunt. Amer. Journ. Physiol. **45**, pp. 197, 231. 1918.
(9) Richards and Drinker. Journ. Pharm. and exp. Ther. **7**, p. 467. 1915.
(10) Bayliss. Proc. Roy. Soc. B. **89**, p. 380. 1916.
(11) Gruber. Amer. Journ. Physiol. **45**, p. 302. 1918.
(12) Gruber. Ibid. **43**, p. 530. 1917.
(13) Vincent and Sheen. This Journal, **29**, p. 242. 1903.
(14) L. Hill. Schäfer's Text-Book of Physiol. **2**, pp. 136, 137.
(15) Cannon and Lyman. Amer. Journ. Physiol. **31**, p. 384. 1913.
(16) Bayliss. This Journal, **26**, p. 173. 1900.
(17) Goltz. Pflüger's Arch. **9**, p. 174. 1874.
(18) Luchsinger. Ibid. **14**, p. 391. 1877. Puelma and Luchsinger. Ibid. **18**, p. 489. 1878.
(19) Roy and Graham Brown. This Journal, **2**, p. 323. 1879.
(20) Barger and Dale. Biochem. Journ. **2**, p. 240. 1907.
(21) Hoskins, Gunning and Berry. Amer. Journ. Physiol. **41**, p. 513. 1916.
(22) Eppinger. Wien. med. Woch. **23**, p. 1414. 1913.
(23) Sollmann and Pilcher. Journ. Pharm. and exp. Ther. **9**, p. 309. 1917.
(24) Brodie. This Journal, **26**, p. 48. 1900.
(25) Cotton, Slade and Lewis. Heart, **6**, p. 223. 1916.
(26) Langley. This Journal, **27**, p. 248. 1901.
(27) Bainbridge and Evans. Ibid. **48**, p. 278. 1914.
(28) Richards and Plant. Journ. Pharm. and exp. Ther. **7**, p. 485. 1915.
(29) Stewart and Rogoff. Ibid. **8**, p. 479. 1916.
(30) Barcroft. The Respiratory Function of the Blood, Cambridge. 1914.
(31) Barcroft and Piper. This Journal, **44**, p. 359. 1912.
(32) Cannon. Journ. Amer. Med. Assoc. **70**, p. 611. 1918.
(33) Dastre and Morat. C. R. Acad. d. Sci. **91**, p. 393. 1880.
(34) Tribe. This Journal, **48**, p. 154. 1914.
(35) Langley. Ibid. **41**, p. 483. 1911.